β-GALACTOSIDASE DETECTION USING A LONG PERIOD GRATING FIBER SENSOR

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Here we report a long period grating fiber sensor used to detect β-Galactosidase in water, an enzyme produced by E. coli that can be used to reveal the presence of the bacteria and their concentration. The advantage of this approach is that β-Galactosidase is produced by a vivid bacterium and its concentration is proportional to bacteria concentration. The purpose of this study is to determine the sensitivity of such sensor. The measurement of β-Galactosidase concentration bases on the long period grating fiber high sensitivity at refractive index modifications of the ambient. Tests proved a good concordance between calculations and measurements.

Keywords: β-Galactosidase, Long Period Grating Fiber Sensor, refractive index detection.

1. Introduction

For many complex systems, ranging from industrial processes, environmental and biochemical applications, the measurement of chemical and biological parameters in various environments is an important issue. In situations as air or water quality control it is extremely important to monitor remotely in real time the chemical compounds (e.g. green-house gases) or biological agents (e.g. toxins, bacteria). This issue is frequent in milk, pork, sheep, poultry meat processing and preservation [1-10]. In circumstances as anaerobic digesters or aquaculture tanks, real-time monitoring of chemical parameters is critical for the economic viability of these facilities. In many occasions, either appropriate technology is not available, or is time consuming, often because they require pre-treatment of the samples. A source of time-consuming is the long period of time between sample collection and results thus cancelling effective corrective actions. Fiber optic sensors technology stands as a promising alternative to standard technologies due to high sensitivity, immunity to electromagnetic interference, is chemically and biologically inert, the small size of components and capacity for in-situ, real-time

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and distributed sensing. A very efficient solution among fiber optic sensors are Long Period Grating Fiber Sensors (LPGFS) with operation based on evanescent light field interaction with the ambient, more accurate than another approach, that using plasmonic resonance [11-13]. Thus, arises the need to design real-time, in-line sensors, able to perform accurate measurements of chemical and biological parameters in time of few minutes. The design of such sensors by our team is described in [14-15]. Detection of Escherichia coli O157:H7 in water, a very dangerous strain, rises big problems to ensure a safety preliminary testing of the sensor. Fortunately there is a solution that can be used also to detect the bacterium, not only during tests. A simulation model of the life cycle / evolution of the bacterium Escherichia coli strain O127: H6 revealed 7 compounds characteristic of the bacterium Escherichia coli strain O127: H6, enzymes that can be used as "markers" of the presence and concentration of the bacterium. They belong to the class of lipopolysaccharide biochemical compounds and perform functions of enzymes and / or antigens being bound to the outside of the membrane of the analyzed bacterium. One of them is β-Galactosidase, the "marker" which was used during our experiments. This is why we studied a detector of β-Galactosidase in water.

2. Materials and Methods

The operation of a sensor detecting β-Galactosidase in water is as it follows (similar to [14]): a fiber interrogator (I-MON 512 USB, Ibsen Photonics, Denmark) injects a wide-band light beam in the long period grating (LPG) fiber, through a fiber circulator (6015-3-APC, Thorlabs, USA). The LPG fiber, with the protective coating stripped in the active area of the LPG, is placed in a sealed enclosure in which the tested solution is pumped through an intake and exits by a drain, through an escape. The solution is in close contact with the LPG fiber cladding (the coating being stripped out). Thus, a transmission spectrum specific to the actual refractive index of the solution is created and the beam leaving LPG is directed back to the fiber interrogator, through the circulator. Inside the interrogator the incoming light beam is directed to a photodetector array, analyzed and measurement results are sent to a PC for further processing. The LPG (from KS Photonics, South Korea) is made of Corning SMF28 fiber, having 60 gratings with a period of 667 μm. The device working wavelength is 1550 nm. The tested solution was β-Galactosidase from Escherichia coli (G4155-1KU, Sigma-Aldrich, USA) in water.

The sensitivity of the method to detect a specific enzyme regardless the presence of other enzymes in the same sample bases on unique spectral refractive index dispersion of each enzyme over a broad (1510-1595 nm) wavelength range.

Several solutions with different concentrations of β-Galactosidase were used during testing. Five samples were prepared: one reference sample containing

distilled water, and four having the following concentrations: 0.05%, 0.1%, 0.5% and 1%.

The liquid volume of a sample was set at V = 5 mL (useful volume of the syringe and consistent with the volume of the inner channel of the sealed enclosure). β-Galactosidase from Escherichia coli is purchased in a solution of concentration C_0 = 50%, therefore to prepare solutions with different concentrations the procedure was as it follows: the volume of solution V_s with concentration C_0 necessary to get a solution with concentration C (0.05%, 0.1%, 0.5% and 1%) was calculated. Denoting by V_g the volume of β-Galactosidase, the following relation can be written:

$$V_g = V \cdot C = V_s \cdot C_0 \tag{1}$$

From this it is immediately deduced:

$$V_s[\mu L] = V[\text{mL}] \cdot 1000 \cdot \frac{C[\%]}{C_0[\%]} \tag{2}$$

The five samples were done by adding distilled water until the desired volume V = 5 mL is obtained. The volume V_g will, of course, be half (C_0 = 50%) of V_s.

The next step is to calculate the refractive index of the solutions with different concentrations. The solution of concentration C will have a refractive index n_s which is between that of water (n_w) and that of β-Galactosidase (n_g), of course depending on the concentration C:

$$n_s = n_w + \left(n_g - n_w\right) \cdot C[\%]/100 \tag{3}$$

The above equation shows a linear dependence of the refractive index of the solution on the concentration. Measurements [16] prove this assertion.

The refractive indices of water (n_w) and of β-Galactosidase (n_g) for the 511 wavelengths of broadband light radiation (1510-1595 nm) produced and used by the fiber optic interrogator were calculated basing on dispersion formulas available on refractive index database website [17].

The transmission spectrum of the Long Period Grating is modified under ambient refractive index variation [18-20]. In the case of a grating with a modulation period Λ the discrete resonance wavelengths $\lambda^{(i)}$ are defined by the equation [18,19,21-29]:

$$\lambda^{(i)} = \left(n_{effco}\left(\lambda^{(i)}\right) - n_{effcl}^{(i)}\left(\lambda^{(i)}\right)\right)\Lambda \tag{4}$$

where n_{effco} is the core effective refractive index and $n_{effcl}^{(i)}$ are the effective refractive index values corresponding to the possible light cladding co-propagating modes. For each $\lambda^{(i)}$ the coupling coefficient between the core and the i^{th} cladding mode, κ_i, is calculated using Bragg resonance wavelength value, the grating modulation period and amplitude values [24-32]. From Eq. (4), the grating characteristic Phase Matching Curves (PMC) are calculated. PMC are parametric curves defining the pairs grating period Λ and Bragg resonance wavelength values

$\lambda^{(i)}$. In Eq. (4) n_{effco} and $n_{effcl}^{(i)}$ are both functions of the refractive index of the ambient.

An improvement of the LPGFS sensitivity may be obtained using a modified version of Eq. (4) [24, 33]:

$$\lambda^{(i)} = \left(n_{effco}\left(\lambda^{(i)}\right) - n_{effcl}^{(i)}\left(\lambda^{(i)}\right)\right)\Lambda + (\kappa_{c-c} - \kappa_{cl-cl}) \quad (5)$$

where $\kappa_{c\text{-}c}$ and $\kappa_{cl\text{-}cl}$ are the self-coupling coefficients of the core mode and the cladding modes, respectively.

3. Results

Five transmittance measurements were performed for the five concentrations of tested solutions. The transmissions depending on the wavelength, for the five solutions were put together in Fig. 1.

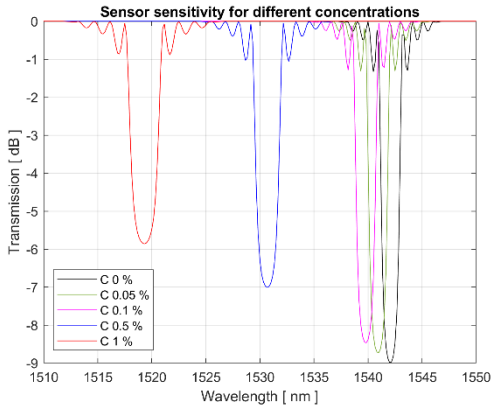

Fig. 1. Sensitivity of the sensor to different concentrations.

Transmission spectra were also calculated basing on Eq. (5) [24, 33]. Both measured and calculated data represent transmissions at 511 wavelength in the range 1510-1595 nm, for five different concentrations. For each concentration dataset was determined by simple algebra the minimum transmission, its position (peak position) and the peak width (half measure full width). Then peak wavelength shift was calculated for each concentration as difference between the peak position at a specific concentration and that of the pure water (concentration 0 %).

The following data were determined: λ_0 (peak position), T_{min} (minimum transmission), w (peak width) and $\Delta\lambda_0$ (peak wavelength shift), all depending on the concentration C of the solution. Calculated data are summarized in Table 1 while measured data are summarized in Table 2.

Table 1

Calculated data

C [%]	λ_0 [nm]	T_{min} [dB]	w [nm]	$\Delta\lambda_0$ [nm]
0	1542.070	-9.000	1.714	0.000
0.05	1540.932	-8.738	1.765	-1.138
0.10	1539.794	-8.477	1.820	-2.276
0.50	1530.689	-7.012	2.200	-11.381
1.00	1519.308	-5.860	2.632	-22.762

Table 2

Measured data

C [%]	λ_0 [nm]	T_{min} [dB]	w [nm]	$\Delta\lambda_0$ [nm]
0	1542.066	-9.083	1.695	0.000
0.05	1540.934	-8.814	1.750	-1.132
0.10	1539.803	-8.555	1.806	-2.263
0.50	1530.681	-7.079	2.185	-11.385
1.00	1519.302	-5.917	2.621	-22.764

A number of 3000 transmission measurements were done for each 511 wavelengths (ranging from 1510 nm to 1595 nm with a step of 0.166 nm). From these data three important parameters were determined: peak position, minimum transmission (both determined by finding the minimum value and position) and peak width (calculated at half amplitude, between maximum and minimum), each parameter comprising 3000 samples. There were done five transmission measurements sets for the five studied concentrations (0%, 0.05%, 0.1%, 0.5% and 1%), thus obtaining five sets of the three parameters. Statistics results are presented in Table 3.

Table 3

Measured parameters statistics

C [%]	λ_0 [nm]		T_{min} [dB]		w [nm]	
	Average	St. dev.	Average	St. dev.	Average	St. dev.
0	1542.066	0.135	-9.083	0.094	1.695	0.006
0.05	1540.934	0.137	-8.814	0.083	1.750	0.006
0.10	1539.803	0.137	-8.555	0.085	1.806	0.006
0.50	1530.681	0.153	-7.079	0.064	2.185	0.007
1.00	1519.302	0.163	-5.917	0.047	2.621	0.008

The peak position λ_0 measurements are displayed in Fig. 2 together with calculated data. The minimum transmission T_{min} measured and calculated data are shown in Fig. 3. Fig. 4 presents peak width w measured and calculated data.

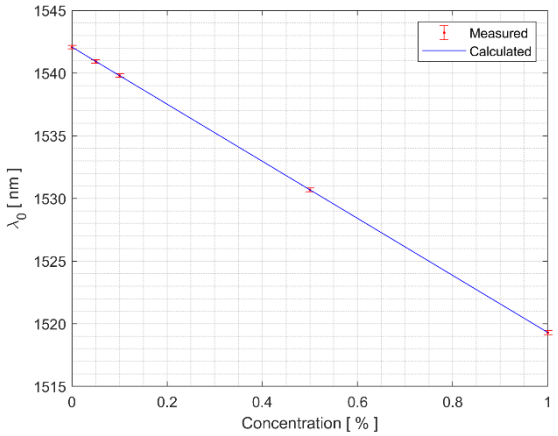

Fig. 2. Peak position λ_0 at different concentrations.

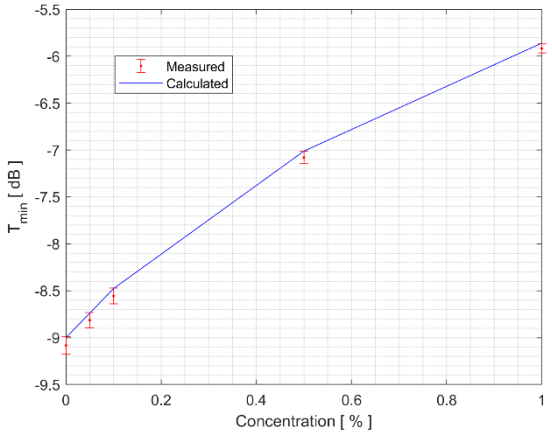

Fig. 3. Minimum transmission T_{min} at different concentrations.

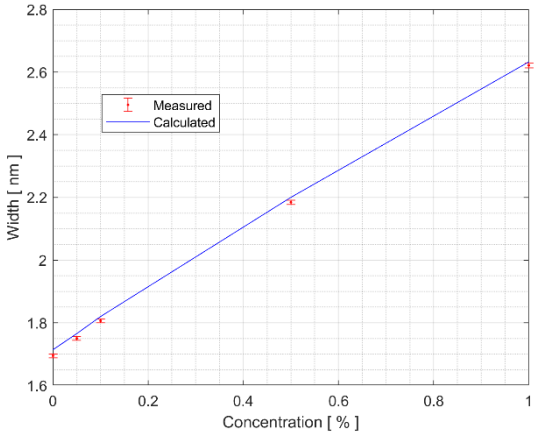

Fig. 4. Peak width w at different concentrations.

4. Discussion

The sensitivity of the sensor was estimated by finding the smallest value of concentration that can be detected and the smallest interval between two different concentrations that can be separately identified (resolution).

As it can be seen from Fig. 2 peak position λ_0 varies quite linear with concentration. A derivative of the concentration with the peak position ($\frac{dC}{d\lambda_0}$) was calculated for the five concentrations and summarized in Table 4, together with its statistics.

Table 4

Derivative of the concentration with the peak position

C [%]	0.05	0.10	0.50	1.00	Average	Std. dev.
$dC/d\lambda_0$ [%/nm]	-0.0441	-0.0442	-0.0439	-0.0439	-0.0440	0.00016509

Notice the linearity of $dC/d\lambda_0$: the standard deviation is 0.00016509 %/nm, which is 0.375% of the average (-0.0440 %/nm). In this situation $dC/d\lambda_0$ can be considered equal to -0.0440 %/nm for the whole concentrations range (0 to 1 %). The smallest value of concentration that can be detected is given by the wavelength sampling step of the interrogator (0.167 nm) multiplied by $dC/d\lambda_0$, giving 0.0073 % (or 73 ppm). The resolution (the smallest interval between two different concentrations that can be separately identified) has the same value since it is given by the same wavelength sampling step of the interrogator (0.167 nm) multiplied by $dC/d\lambda_0$, which is almost constant.

The temperature influences the measurement only by modification of the refractive indices of the solution components. In this study this influence wasn't taken into account, but we will do it in the future.

5. Conclusions

A long period grating fiber sensor for detection of β-Galactosidase in water was successfully tested, proving a good accuracy. The β-Galactosidase being a marker of the presence of a vivid E. coli bacterium a sensor detecting this enzyme provides a convenient solution for an E. coli detector.

As a further development we aim to redesign the LPG to detect the specific change of the refractive index of other environments (as milk, for instance) and even finding markers for other bacteria (like Klebsiella) in a specific environment (water, milk, and so on).

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