

Fiber optics biosensor fabricated for measuring the growth rate of *Escherichia coli* K-12 in the Aqueous medium

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The aim of this study is to fabricate a Refractive Index TFOBS in order to determine the growth rate of *Escherichia coli* K-12. Fiber optic biosensors have been widely investigated because of their potential sensitivity, detection speed, and adoptability to a wide variety of assay conditions. Methods of cladding removal and tapering enable evanescent field sensors to be formed from plastic optical fibers (POF). It is known that tapered fibers exhibit excellent sensing capabilities due to the existence of a strong Evanescent field (EVF) in the tapered region [1].

Immobilization of the sample on tapered region using a polymeric substance, affects the EVF's magnitude by absorbing [2], scattering [3], changing the refractive index of the adjacent cladding [4], or by Surface Plasmon resonance [5] and fluorescence phenomena [6]. In this study we examine the effect of changing the refractive index in the tapered region of the fiber. Increasing the refractive index of the medium surrounding the tapered fiber results in a decrease in the number of modes to be propagated and hence this will considerably change the intensity of the transmitted light [4].

Single mode fiber was tapered with a Co2 laser (SYNARD, 48-1SAL), using Heat-pulling method. Tapered fibers were fabricated with waist diameters of 6–8 μm and of 4-8 μm waist length (Fig 1).

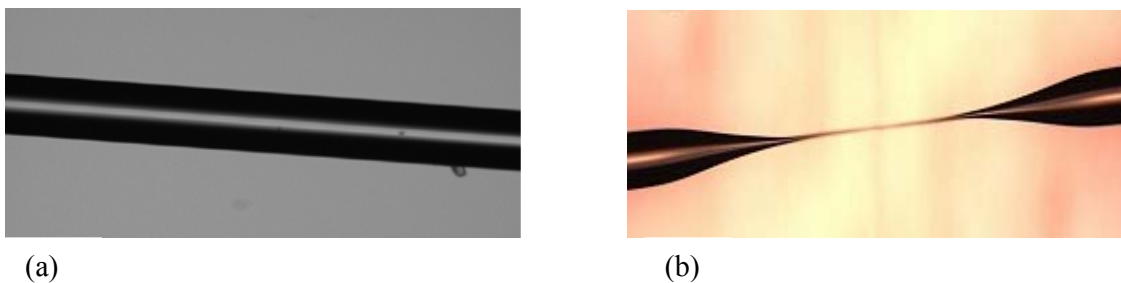


Fig1. Image of optical fiber (a) before tapering, (b) after tapering

The tapered section of each fiber was washed with 70% ethanol (v/v) containing 1% HCl (v/v), then rinsed with deionized water at room temperature. The fibers were dried in micro biological safety cabinet for thirty minutes.

E. coli K-12 was grown under optimal conditions in Nutrient Broth at 37 °C. A 14 to 18-hour culture was used for experimental purposes to mimic environmental conditions. The cell suspension was centrifuged and the supernatant removed. The pellet was resuspended in sterile phosphate-buffered saline-A (PBSA) to obtain an *E. coli* concentration of approximately 10^8 colony-forming units (CFU)/ml as determined by 0.5 McFarland standard and spectrophotometric assays [7].

Poly-L-lysine is commonly used to immobilize negatively charged molecules such as bacteria and DNA to glass surfaces. It has positively charged amino-groups that can bind to the negatively charged silica surface through an ionic binding and immobilizes in monolayer form on the surface. For this purpose a solution of 0.1 % Poly-l-lysine (Sigma, P8920) was placed on each tapered fiber and allowed to evaporate over night [8].

The experimental set up in (figure2) consists of an Edge Light Emitting Diode (ELED), with the central wavelength, FWHM, and power equal to 1547 nm, 79.6 nm, and 50 μW , respectively. The light of ELED which passes a dual stage isolator and a 2x2 coupler arrives into the taper fiber sensor standing in the Fiber holder. Then photodiode number 2 (PD2) detects the light. The reflected light of 2x2 coupler is observed by photodiode number 1 (PD1). Then labview software normalizes the Data from two similar detectors delivered by A/D convertor.

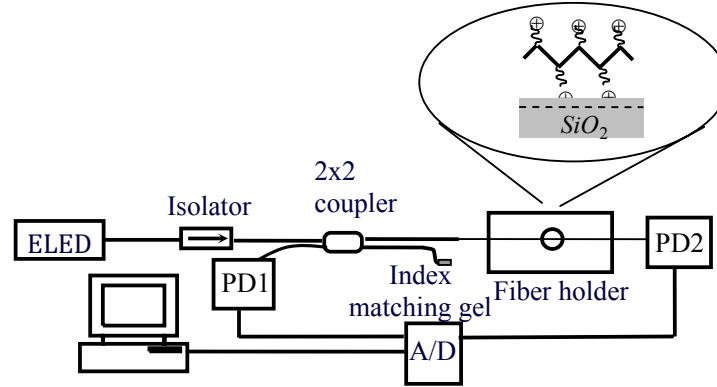


Fig 2 .Instrumentation set up to measure bacterial growth rate.

Cultivated *E. coli* was contacted with the coated tapered fiber for 30 min, the unattached cells were removed and the fiber rinsed gently in PBSA twice. Growth medium (1 g/L glucose, 0.1 g/L yeast extract in 100mM PBSA) was added into the well surrounding the tapered section. Intensity of the light passing through detectors was measured for eight hours at room temperature.

The refractive index of the *E. coli* K-12 sample is typically 1.384 [9], the growth medium 1.33 and those of the original cladding and core are 1.458 and 1.46, respectively. As can be seen in (fig.3), the growth of the bonding bacteria increases the fiber diameter slightly as well as changing the refractive index at the taper surface. While immobilized *E. coli* grows, the effective refractive index of the medium, surrounding the fiber increases, as a result the reduction in the number of modes propagating in the tapered region cause to decrease the transmitting intensity.

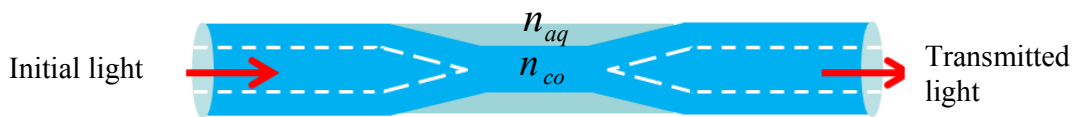


Fig 3 .Instrumentation set up to measure bacterial growth rate.

Consider the transmitted power at zero time (shortly after inoculation) as I_0 . At time, t , when some growth has occurred on the surface of the taper, let the transmitted light be represented by

$$I = -\alpha X^\beta \quad (1)$$

Where X is surface concentration (per unit area).The parameters α and β are characteristics of the taper and optical properties of the bacteria that is grown on the taper surface. From Eq.(1), one can obtain

$$\text{Ln}\left(\frac{I}{I_0}\right) = -\beta \text{Ln}\left(\frac{X}{X_0}\right) \quad (2)$$

Where subscript 0 refers to inoculum condition. Assuming exponential growth under substrate excess conditions which results in constant specific growth rate, Eq. (2) reduces to

$$\ln\left(\frac{I}{I_0}\right) = -\beta\mu_m t \quad (3)$$

Where μ_m maximum specific growth rate and t is is time. Eq.(3) suggests that a plot $\ln(I/I_0)$ versus time will yield a straight line with the slope proportional to specific growth rate, $\beta\mu_m$. Growth rate of E. Coli K12 was determined via variation in the EVF intensity and compared to parallel biological procedure entitled Pour Plate Method.

References

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