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Plastic optical fiber immunosensor for fast detection of sulfate-reducing bacteria

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ABSTRACT

Sulfate-reducing bacteria (SRB) are considered one of the oldest forms of microorganism that date back about 3.5 billion years and are common in anaerobic environments such as oil wells. SRB, in their metabolic process, produce hydrogen sulfide that contaminates the oil and is extremely toxic to humans, besides being one of the main causes of corrosion in the pipes. This paper presents a fast response-time biosensor, made of 1-mm diameter plastic optical fiber (POF) to detect SRB in production water. The physical principle for the operation is the interaction of the measurand with a curved waveguide based on the changes of the refractive index of the medium. The POF probes were functionalized with antibody anti-SRB and tested with *Desulfovibrio alaskensis* concentrations of 10^4, 10^6 and 10^8 MPN/mL (most probably number per mL). The optoelectronic setup consists of an 880 nm LED connected to a U-shaped probe. The system responded in about 30 min for concentrations of 10^4, 10^6 and 10^8 MPN/mL, encouraging an optimization of the sensitivity to read concentration of 10^3 MPN/mL or smaller. To the best of our knowledge, this is the first sensor to date capable to detect SRB with such small-time response.

1. Introduction

Sulfate-reducing bacteria (SRB) are microorganisms that obtain energy by oxidizing organic compounds or molecular hydrogen (H_2) at the same time that reduce sulfate (SO_2^-) to produce hydrogen sulfide (H_2S). Since SRB are anaerobic, they use sulfate rather than oxygen as a form of respiration. SRB, considered one of the oldest forms of microorganism that date back about 3.5 billion years, are common in anaerobic environments such as oil wells.

In oil and gas exploration and production (E&P) industry, SRB can cause corrosions as they can produce biofilms in the internal surface of oil pipes and tanks, particularly when sulfate is present in production water or in the petroleum. The metal structures exposed to sulfate-containing water allows the SRB to produce hydrogen sulfite which, in presence of water, reacts producing sulfuric acid (H_2SO_4) which corrodes steel [1]. SRB also contaminate fuel gas and oil with H_2S. Furthermore, hydrogen sulfide is extremely toxic to humans as it easily escapes from water and oil and may accumulate in closed locations. It is easily recognized by its distinctive rotten egg odor, but in higher concentrations our sense of smell is anesthetized, which can be another serious problem because this gas can cause shock, convulsions, inability to breathe, rapid unconsciousness, coma and death. Effects can occur within as little as a single breath, according to the Occupational Safety and Health Administration (OSHA). Apart from its health hazards, hydrogen sulfide is explosive and flammable. For these reasons H_2S presence in crude oil is controlled, and cannot exceed 5 ppm, as ruled by the U.S. Federal Energy Regulatory Commission (FERC) [2].

Although hydrogen sulfide is a naturally occurring component of crude oil and natural gas, it is also largely produced by SRB, therefore the oil contents of H_2S also indicates the presence of these bacteria. For SRB controlling, huge amounts of bactericide are spent yearly to control oil contamination.

Nowadays the amount of SRB in crude oil can only be measured by analytic laboratory techniques by media preparation and bacteria culture. These procedures can only be made inshore and it takes about one month to collect samples offshore, taking them to an inshore laboratory and producing a positive response of SRB presence. Bacteria are collected in production water samples as it is impossible to isolate them from crude oil. These bacteria are not normally isolated because of their slow growth since the colonies appear after more than three days of incubation in a specific or selective growth medium [3]. For these reasons, the quantification of SRB is important in E&P industry, not only for controlling H_2S contamination but also for corrosion avoidance.

About 220 species of sulfate-reducing bacteria are known [4]. Among the orders of SRB, the families of *Desulfobacterales,*

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Desulfovibrionales and *Syntrophobacterales* form the largest group, accounting for 23 genera. In this work, we tested the sensor with *Desulfovibrio alaskensis*, isolated from the petroleum produced in the Campos Basin oil field in Brazil. This bacterium is considered an indicator of SRB contamination, as in most cases *D. alaskensis* is present among another species.

In this study, we developed and characterized a U-shaped plastic optical fiber (POF) biosensor for in situ and fast detection of sulfate-reducing bacteria. The biosensor is based on the immunocapture technique by direct immobilization of an antibody to the optical fiber surface. This way the specific bacteria of interest concentrates at the fiber surface, whereas other bacteria eventually present in the sample do not interfere with the measurements.

2. Sensing principle

An optical fiber biosensor normally detects the presence of the cells around the fiber by refractive index (RI) variation such as in our previous works [5–7]. Some other studies detect the fluorescence emitted by the cells [8,9], or by enzymatic reaction [10].

The use of RI as a sensing parameter is based on the fact that bacteria, due to their intracellular liquid and organelles, present an RI of approximately 1.39, slightly higher than that of pure water (n = 1.334) [6]. Due to this small difference, it is not possible to detect bacteria simply by measuring the RI of a water containing a reasonable concentration of bacteria, say 10^5 or 10^4 colony forming units (CFU), since the average RI of the mixture is not different to that of pure water, except at the fifth or sixth decimal position. Therefore, in order to detect bacteria, one has to concentrate them around the fiber by covalently capturing the cells by a specific antibody around the sensitive region of the fiber. In this way, all bacteria of that specific species will be captured and fixed around the sensitive part of the sensor producing an increase of the RI that will be detected by the sensor in a technique known as immunocapture.

The principle of RI sensing is based on the evanescent wave (EW). When light propagates through an optical fiber, the modes are bounded inside the core by the phenomenon known as total internal reflection (TIR). All light rays incident at the core-cladding interface with angles greater than the critical angle will suffer TIR. This angle is given by:

$$\theta_c = \sin^{-1}\left(\frac{n_{cl}}{n_{co}}\right)$$

where n_{cl} is the RI of the cladding and n_{co} is that of the core. In our case, as it will be shown below, we use uncladded fibers so that the cladding of the fiber is in reality the outside medium.

EWs are formed on the other side of the guiding core, when waves traveling in a medium undergo TIR at its boundary because they strike it at an angle greater than the critical angle. At a fiber bend, the propagation conditions alter because light rays propagating close to θ_c will reach the core-cladding interface in an angle smaller than θ_c and will therefore refract to the cladding instead of reflecting by TIR and then part of the power is lost into the cladding or to the outside medium in case of a claddless fiber. The amount of lost modes is proportional to the outside medium refractive index, therefore the greater the outside RI, the more light will be lost to the outside medium. In this way, the solution's RI will dominate the output power of light that is guided by the fiber.

In presence of scattered bacteria at the fiber surface, the local outside RI will increase and, therefore, any incident light ray that hits a bacterium, will refract to the outside medium if it is close to the critical angle.

All other light rays that do not hit any bacterium will suffer TIR and will therefore reflect back to the core. As the bacteria is scarcely distributed along the fiber surface, the majority of high modes rays will behave normally, bouncing side to side inside the core until eventually reach the fiber end and is detected by the photodiode. In this way, the detected light power will be inversely proportional to the number of bacteria bound to the fiber surface. This concept is in consensus with the results of a recent work of Rodrigues et al. [11] that applied the software BeamProp® based on the Beam Propagation Method (BPM) to simulate the light propagation inside a U-shape optical fiber with simulated bacteria scattered alongside the waveguide.

3. Materials and methods

3.1. Probe fabrication

The sensor is a U-shaped probe made of multi-mode SI-POF (step-index plastic optical fiber) Mitsubishi Eska GH4001. The POF has a poly (methyl methacrylate) (PMMA) core of 980 μm in diameter and a fluorinated polymer cladding with 10 μm in thickness. The RI in the visible range of interest is about 1.49 for the PMMA core and 1.41 for the fluorinated polymer at the cladding.

For sensor fabrication, the fiber was cleaved into several 10-cm long sections and both end surfaces were polished for a better light coupling. Following that, the fiber sections were rinsed with deionized water and blow-dried with nitrogen. Then, the POFs were heated at about 70 °C and bent around a mold to produce U-shaped probes with 25 mm length and 9 mm bent diameter. The sensors were tested under different RI, in order to calibrate their sensitivity, and further functionalized with antibodies. The functionalizing protocol and results are presented in the next sessions.

There are several studies dealing with sensitivity of U-shaped probes for RI sensing. In [12], for instance, the authors tested several U-shaped tapers in order to find the ones with best sensitivity. They found that the 600 μm waist diameter and 2.0 mm macro-bending curvature probe presented the best sensitivity.

In [13] it was tested several U-shaped POF probes for the optimal probe design, concentrating on the bend diameter. The tests were performed with several fiber diameters but they found the 3-mm bent diameter the better one, regardless the fiber diameter.

Although sensitivity seems to be a critical parameter in order to detect such small amount of bacteria, say, 10^4 CFU/mL, another constraint is the ease in machinability and handling in order to enable a viable alternative for mass production. Therefore, we maintained our former and simple dimensions, 1 mm fiber diameter and 9 mm curvature diameter, despite the slightly smaller sensitivity obtained.

For the antibody immobilization, we followed a protocol described by [14] where the authors modified a PMMA substrate with primary amino groups appropriate for immobilizing different types of biomolecules. In our previous experiments mentioned above, we applied the procedure directly over the POF surface, that is, on the cladding. However, the POF makers do not disclose the chemical formula of the cladding material; just inform that it is a fluorinated material. Since we had in previous developments a low sensitivity to Escherichia coli at 10^4 CFU/mL after the functionalization, we wondered that the immobilization procedure was not appropriated for the cladding material.

In order to check for the presence of PMMA radicals in the cladding, we performed a FTIR Spectroscopy for Attenuated Total Reflectance (ATR) with a MiracleA (Shimadzu Corporation, Japan) in the pristine POF [15], which result is shown in Fig. 1 (top). In sequence, we performed the same test on a 1-mm diameter cladless POF (Sojitz Europe plc), shown in Fig. 1 (bottom). Notice that the peak corresponding to the PMMA ($C_5O_2H_8$), at 1724.44 cm^{-1} is present in Fig. 1 (bottom) (circled by a dotted line), whereas it is very small in Fig. 1 (top). This means that the cladding of the GH 4001 fiber does not contain much PMMA in its molecule.

Another test was performed with confocal microscopy after functionalization and antibody immobilization in both cladded and cladless fibers. The antibodies were marked with Alexa Fluor 488 (Sigma, Brazil), a bright, green-fluorescent dye with excitation matched to the