U-shaped, double-tapered, fiber-optic sensor for effective biofilm growth monitoring

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Abstract: To monitor biofilm growth on polydimethylsiloxane in a photobioreactor effectively, the biofilm cells and liquids were separated and measured using a sensor with two U-shaped, double-tapered, fiber-optic probes (Sen. and Ref. probes). The probes’ Au-coated hemispherical tips enabled double-pass evanescent field absorption. The Sen. probe sensed the cells and liquids inside the biofilm. The polyimide–silica hybrid-film-coated Ref. probe separated the liquids from the biofilm cells and analyzed the liquid concentration. The biofilm structure and active biomass were also examined to confirm the effectiveness of the measurement using a simulation model. The sensor was found to effectively respond to the biofilm growth in the adsorption through exponential phases at thicknesses of 0–536 μm.

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disadvantages faced by conventional floc-based systems and often achieve higher effluent and industrial wastewater because biofilm-based technologies can overcome several high-quality biofilms [5]. Appropriate biofilms with high activities are used to treat municipal and drugs [4]. However, effective bioremediation and biohydrogen production also require undesirable effects occur because the EPSs in biofilms increase their resistance to antibiotics greater concentrations of antibiotics than are necessary to treat bacterial suspensions [3]. These pulmonary disease, and so on [2]; in the water industry, once biofilms are established, their organ system, biofilms can cause periodontal disease, dental caries, chronic bronchitis, [1]. The presence of a biofilm can be detrimental or beneficial. For example, in the human organ system, biofilms can cause periodontal disease, dental caries, chronic bronchitis, pulmonary disease, and so on [2]; in the water industry, once biofilms are established, their toxic secretions can lead to severe infections that are difficult to treat, often requiring much greater concentrations of antibiotics than are necessary to treat bacterial suspensions [3]. These undesirable effects occur because the EPSs in biofilms increase their resistance to antibiotics and drugs [4]. However, effective bioremediation and biohydrogen production also require high-quality biofilms [5]. Appropriate biofilms with high activities are used to treat municipal and industrial wastewater because biofilm-based technologies can overcome several disadvantages faced by conventional floc-based systems and often achieve higher effluent

1. Introduction

A biofilm consists of liquids and layers of cells embedded in a matrix consisting of a conglomeration of different types of biopolymers (extracellular polymeric substances, EPSs) [1]. The presence of a biofilm can be detrimental or beneficial. For example, in the human organ system, biofilms can cause periodontal disease, dental caries, chronic bronchitis, pulmonary disease, and so on [2]; in the water industry, once biofilms are established, their toxic secretions can lead to severe infections that are difficult to treat, often requiring much greater concentrations of antibiotics than are necessary to treat bacterial suspensions [3]. These undesirable effects occur because the EPSs in biofilms increase their resistance to antibiotics and drugs [4]. However, effective bioremediation and biohydrogen production also require high-quality biofilms [5]. Appropriate biofilms with high activities are used to treat municipal and industrial wastewater because biofilm-based technologies can overcome several disadvantages faced by conventional floc-based systems and often achieve higher effluent
quality [6]. In particular, in the biohydrogen field, biofilm attachment is considered to be a highly efficient method of hydrogen production by photosynthetic bacteria (PSB) [7]. Biofilm, therefore, has attracted intense interest for its potential advantages, including its high conversion yield and avoidance of biomass–liquid separation, as well as its dual functions of wastewater biodegradation and hydrogen production. Although biofilm use is promising, the performances of photobioreactors using PSB are still very poor due to the current lack of effective and accurate biofilm thickness measurement and control [8].

Online monitoring of biofilm growth is very important for controlling biofilm thickness and improving biofilm activity to enhance bioreactor performance. However, it is difficult for traditional sensors (optical density, microelectrode, electrochemical, and ultrasound biofilm sensors) to effectively monitor biofilm growth. The main reason for this difficulty is that the designed sensors are not easy to install in bioreactors and exhibit low accuracy because they can scarcely distinguish between the effects of various factors (i.e., the biofilm biomass and liquid) on the biofilm thickness since they measure only the output signal [9–11]. Furthermore, although fiber-optic evanescent wave (FOEW) sensors have attractive properties because of their microstructures, easy installation, corrosion resistance, immunity from electromagnetic interference, and good biocompatibility, the fiber sensors designed thus far have shown narrow detection limits (the maximum measurement range observed thus far was only from several tens of micrometers to 120 μm) [8]. Such narrow measurement ranges do not satisfy the application requirements because, in the H₂ production, CO₂ adsorption, and microbial fuel cell industries, the biofilm thickness can be up to about 180 μm when a support material with appropriate surface pits or grooves is used [12,13]. Furthermore, it is well known that FOWE sensor sensitivity depends on the attenuation of the evanescent waves [14]. The amount of attenuation is affected by the evanescent wave parameters, i.e., the effective intensity, decay coefficient, penetration depth, and optical path length on the unclad fiber surface. These parameters further depend on the sensing region diameter and length, the shapes of the unclad fiber region (taper, U, C, or D-shaped) and fiber end region (hemispherical, triangular, or wedge-shaped), and the surface roughness [15]. Thus, the sensitivities of traditional FOWE sensors have remained low because of inappropriate evanescent wave parameters. Hence, to effectively monitor biofilm growth in a bioreactor, it is important to create an easily installable, high-sensitivity, fiber-optic sensor with a large measurement range.

Therefore, in this work, we developed an online U-shaped FOWE sensor with a double-tapered microstructure using etched fiber probes to enhance the sensitivity and measurement range in biofilm growth monitoring. A model was developed to demonstrate the biofilm thickness measurement principle. We examined the probes’ optical transmission characteristics, temperature responses, and sensitivities and also checked the response speed of a reference (Ref.) probe coated with a prepared polyimide–silica hybrid film (PSHF). Photobioreactors with different hydrophilic polydimethylsiloxane (PDMS) groove depths were fabricated. The biofilms were cultured with both continuous and intermittent supplies of a synthetic medium and were monitored using the designed sensor. Furthermore, the biofilm structures and active biomasses in the biofilms were checked, and the detection limit and accuracy of the proposed sensor were analyzed.

2. Materials and methods

2.1 Biofilm sensor configuration

The two probes for the sensor were made from graded-index optical fiber (FiberHome Technologies, China) with a core diameter of 105 μm ± 3.5 μm, cladding diameter of 125 μm ± 3 μm, coating diameter of 245 μm ± 10 μm, numerical aperture of 0.22 ± 0.02, and SiO₂/GeO₂ and SiO₂ as the core and cladding materials, respectively. To enhance the evanescent field intensity and decay coefficient of and the optical path length on each unclad fiber surface, one end of each fiber was coupled to a SMA905 connector, and the other end was polished into a
hemisphere as shown in Figs. 1(a), 1(b), and 1(d) (the hemisphere of each fiber end was processed by the Beijing Glass Research Institute R&D Center, China); the hemispherical end of each fiber was coated with a Au film about 500 nm thick by a magnetron ion sputtering machine (JGP-450, Shenyang Scientific Instrument Development Center of CAS, China) to improve the light reflection and obtain double-pass evanescent field absorption [16]. Thereafter, chemical etching was employed, and the sensing region was prepared as previously reported by Zhong et al. [17].

Before etching, the jacket of a normal 50-mm-long fiber was removed to prepare each probe, and in both cases the prepared hemispherical end of the fiber was coated with candle oil to avoid corrosion. Each bare fiber was fixed to a U-shaped Teflon holder with a 6 mm radius of curvature in the bend. Each fixed fiber was inserted vertically into a Teflon container (with an inner diameter and wall thickness of 30 mm and 2 mm, respectively) and etched using a hydrofluoric acid/water mixture (0.015 mol/L) at 40 °C. To prevent the volatilization of the hydrofluoric acid, fluorosilicone oil with a density of 0.963 g/cm$^3$ was added dropwise to and floated on the etchant prior to etching. In this study, the oil was also used to prepare the biconical tapered fibers by placing each uncoated fiber vertically in the etchant [18].

After etching, the etched fiber regions were used as the sensing regions. The surface roughness of the etched fibers, $2\delta/\Delta$, was 0.3, as shown in Fig. 1(c), where $\delta$ and $\Delta$ are the average pit depth and diameter, respectively, as defined by Zhong et al. [15]. The fibers were etched rough mainly to enhance the evanescent field intensities, penetration depths, and decay coefficients on the fiber surfaces as well as the stability of the modified film on the unclad fiber of the Ref. probe [15,19]. The structures of the sensing regions are shown in Figs. 1(a) and 1(b); the waist and button diameter of the double-tapered regions were 105 $\mu$m and 25 $\mu$m, respectively. This double-tapered sensing region structure can enhance the evanescent field penetration depth and sensor measurement range [20].

As shown in Fig. 1(a), one of the probes had an exposed fiber core that could sense the cells and changes in the liquid inside the biofilm. Herein, this probe is called the sensor (Sen.) probe. To measure the biofilm growth effectively and accurately, i.e., to eliminate the effects of liquid-phase changes on the thickness measurements, the second fiber core was coated with a PSHF to fabricate the Ref. probe. The PSHF mainly consisted of a polyimide–silica hybrid sol (PSHS). The Ref. probe was produced as follows. (1) The PSHS was prepared as previously reported by Zhong et al. [21]. (2) The PSHS was then coated onto a thoroughly washed etched fiber region using a dip coater and drying it at 200 °C for 600 min; the average thickness of the PSHF coating was about 6 $\mu$m along the double-tapered regions of the fiber. The configuration
of the completed Ref. probe is shown in Fig. 1(b). The surface morphology of the coated PSHF fiber sensing region was obtained using an SEM, as shown in Figs. 1(f) and 1(g). The SEM image (see Fig. 1(g)) indicates that the prepared PSHF formed a porous membrane with an average pore diameter of 0.32 \( \mu \text{m} \). Furthermore, to compare the pore diameter of the prepared PSHF to the size of the PSB \textit{Rhodopseudomonas palustris}, CQK 01 strain, the PSB cell size was determined by examination with an electron microscope [21]; a negative-staining electron microscopic image is shown in Fig. 1(h). By comparing Figs. 1(g) and 1(h), it can be seen that the PSB cells are much larger than the PSHF holes (normal cells have an average length of 4.3 \( \mu \text{m} \) and diameter of 2.2 \( \mu \text{m} \)); thus, the fabricated membrane can be used to separate PSB from bacterial suspensions.

2.2. System operation and measurement methods

Figure 2 shows a schematic illustration of the biofilm photobioreactor (BPBR) biohydrogen production and measurement systems. The flat-panel BPBR was fabricated from polymethyl methacrylate with a working volume of 110 \( \times \) 50 \( \times \) 10 mm\(^3\) and was separated into three regions: a uniform flow region (UFR), useful bioreactor region (UBR), and discharge flow region (DFR). The UFR and DFR were employed to reduce the effects of fluid fluctuations and the fluid shear force on the initial cell adhesion and biofilm growth. In the UBR, a 800-\( \mu \text{m} \)-thick PDMS membrane was fabricated [22], which was made hydrophilic by irradiating it with vacuum ultraviolet (UV) radiation (UER20–172V, Ushil Electric, Japan) for 30 min, because UV/ozone exposure of PDMS causes hydroxyl and a hydrophilic silica-like layer to form [23]. In this work, the PDMS had two functions. Firstly, it was used as the support material for biofilm growth; secondly, a membrane with PDMS grooves was used to fix the fiber sensors (I–III in Fig. 2) because of its flexibility, chemical resistance, and optical transparency. In this study, the \textit{R. palustris} CQK 01 strain (PSB), its culture (continuous flow culture), and its synthetic medium were the same as those described in previous literature [8], except that the flow rate of the synthetic medium was changed to 30 mL/h.

The online biofilm measurement system consisted of the fibers, the Sen. and Ref. probes, an optical filter, a light source, the couplers, the Y-couplers, and an optical power meter. The Y-couplers, which had 95:5 splitting ratios (defined as the ratio of the light intensity in path 1 to that in path 2 in Fig. 2), were purchased from Beijing Glass Research Institute R&D Center, China. The performance parameters of the broad-bandpass filter, light source, and power meter were the same as those reported by Zhong et al. [8]. To detect the temperature in the BPBR, a fiber Bragg grating (FBG) temperature sensor was employed because of its microstructure, corrosion resistance, fast response, and high resolution. The FBG measurement system consisted of the FBG sensor (Bragg wavelength of 1549.28 nm), full spectral scanning (1510–1590 nm) equipment, and an FBG interrogator (SM125-500, Micron Optics Inc.) with high accuracy (1 pm). An optical microscope system (IX81, Olympus, Japan) with a resolution of \( \pm 1 \mu \text{m} \) was used to monitor the biofilm thickness online. Further details on biofilm thickness measurement using optical microscopy have been reported by Bakke et al. [24].
In this work, the surface morphologies of the biofilms were checked using an environmental SEM (ESEM, Quanta200, FEI, USA). Furthermore, the active biomass in the biofilm was examined using confocal laser scanning microscopy (CLSM) as follows: 1) the mature biofilm was sliced into four layers using an HM 505E Cryostat Microtome; 2) the sliced biofilms were stained using SYTO 63 (Molecular Probes, Carlsbad, CA); 3) CLSM (Leica TCS SP5 Confocal Spectral Microscope Imaging System, Mannheim, Germany) was employed to visualize the active biomass in the prepared biofilm samples at 5 μm sampling intervals, and the fluorescence of SYTO 63 was detected via excitation at 633 nm and emission at 650–700 nm.

2.3. Biofilm thickness measurement principle

To establish the sensor’s working principle, we developed a theoretical model, which is presented herein. It is well known that, for an evanescent wave fiber-optic sensor, the transmission of light through an absorbing medium in a fiber can be described by the Lambert–Beer law,

$$I_{\text{out}} = I_{\text{in}}' e^{-\xi L}$$

(1)

where $I_{\text{out}}$ is the output light intensity, $I_{\text{in}}'$ is the effective light intensity incident from the fiber-optic light source, $\xi$ is the decay coefficient of the evanescent waves in the external environment, and $L$ is the length of the unclad (sensing) fiber region. In our previous study on the effects of surface roughness on the light transmission properties of fibers [15], we discovered that if an unclad fiber has a rough surface, some of the light rays incident upon the fiber–medium interface will no longer be totally reflected; instead, they will be attenuated by the interface via scattering and refraction. Thus, the loss of transmitted light does not result from evanescent wave absorption by the surrounding media; instead, the surface roughness prevents some of the light from being retained in the optical signal. Thus, compared to normal fibers with smooth surfaces, the intensity loss can be perceived as a change in the numerical aperture or $U_{\text{max}}$ [25]. Herein, the loss is expressed as a decrease in $U_{\text{max}}$; hence, $I_{\text{in}}'$ for fibers with rough surfaces can be written as [15]

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where $U_i$ is the incident angle of light rays on the end of the fiber and ranges from 0 to $U_{\text{max}}$; $\delta$ and $\Delta$ are the average pit depth and diameter, respectively, for the rough fiber surface, as shown in Fig. 1(e); $y$ is the fiber radius in the thinned region, i.e., the distance CD in Fig. 3; and $I_{\text{in}}$ is the incident light intensity in a normal fiber with a smooth surface [15].

When the sensing regions of the U-shaped fibers in this study were formed into tapered structures, their decay coefficients $\xi$ were corrected as follows [26, 27]:

$$
\xi = \left[ \xi_{\text{eff}}(n) \right]_{\text{outer,1}} + \left[ \xi_{\text{eff}}(n) \right]_{\text{inner,1}},
$$

where $\left[ \xi_{\text{eff}}(n) \right]_{\text{outer,1}}$ and $\left[ \xi_{\text{eff}}(n) \right]_{\text{inner,1}}$ are the evanescent field absorption at the outer and inner surfaces of the bent core, respectively. $\left[ \xi_{\text{eff}}(n) \right]_{\text{outer,1}}$ is given by

$$
\left[ \xi_{\text{eff}}(n) \right]_{\text{outer,1}} = \frac{\alpha n K'_{(R,L,\beta,r,\delta/\Delta)\text{outer}}}{r(n^2_{\text{eff}} - n^2_1)},
$$

where $\alpha$ is the bulk decay coefficient of the external environment; $\lambda$ is the operational wavelength of the light source; $n_\text{eff}$ is the refractive index of the etched-fiber core at radius $x$,

$$(n_\text{eff}) = n_\text{max} \left[ 1 - \frac{n^2_\text{max} - n^2_1}{n^2_\text{max}} \left( \frac{y}{R} \right)^\gamma \right]^{1/2},$$

where $y < R$, $n_\text{max}$ is the refractive index of the fiber core axis [i.e., at $R = 0$], and $n_1$ is the refractive index of the fiber at taper waist radius $R$; $r$ is the fiber bending radius, i.e., the distance AO in Fig. 3; and $n$ is the refractive index of the external environment. The parameter $K'_{(R,L,\beta,r,\delta/\Delta)\text{outer}}$ is a function of $R$, the taper region length $L$, the taper angle $\beta$, $r$, and $\delta/\Delta$ and is given as follows:

$$
K'_{(R,L,\beta,r,\delta/\Delta)\text{outer}} = \int_0^L \int_0^{2\pi} \int_0^{\psi_1} \int_0^{\psi_2} \frac{\cos^3(\theta - \beta)}{4\pi} \frac{[1 - n^2_1 \cos^2(\theta - \beta)] [n^2_\text{eff} \sin^2(\theta - \beta) - 1]^{1/2}}{\theta} \sin(\theta - \beta) \cos(\theta - \beta) \sin(\theta - \beta) d\theta d\psi dl',
$$

where $\psi_1$, $\psi_2$, $\theta$, $\beta$, and $\Delta$ are the variables defined in the figure. The other variables are defined in the text.

Fig. 3. Schematic diagram of light transmission in tapered fiber with rough surface (NR: normal region; ER: etched region; SP: surface pit; the subscripts “re” and “in” on $I$ denote reflected and incident light, respectively).
where \( L' \) is the distance along the axis between the taper region and point A, as shown in Fig. 3, and ranges from \( 0 \) to \( L \); \( \theta \) represents the angle that a guided ray makes with the normal to the core–cladding interface in the straight fiber region and is transformed into the angles \( \varphi \) and \( \psi \) at the outer and inner surfaces of the sensing region, respectively; and \( h \) is the distance between point A and B. The integral of \( \theta \) around the outer surface ranges from \( 1 \) to \( 2 \), which can be expressed as

\[
\varphi = \arcsin \left[ \frac{(r-h)n_2}{(r+R-L'\tan \beta)n_1} \right] \tag{7}
\]

and

\[
\psi = \arcsin \left( \frac{r-h}{r+R-L'\tan \beta} \right) - \arctan(2\delta/\Delta), \tag{8}
\]

where \( n_2 \) is the refractive index of the fiber cladding.

Similarly, the evanescent field absorption \( [\xi_{\text{eff}}(n)]_{\text{inner}} \) at the inner surface of the bent core is given by

\[
[\xi_{\text{eff}}(n)]_{\text{inner}} = \frac{\alpha \lambda n_1 K'_{(R,L,\beta,\epsilon,\delta,\Delta)\text{inner}}}{r(n_2^2-n_1^2)}, \tag{9}
\]

where

\[
K'_{(R,L,\beta,\epsilon,\delta,\Delta)\text{inner}} = \int_0^{L'} \int_0^{L'\tan \beta} \frac{\cos^2(\theta-\beta)}{[1-n_2^2 \cos^2(\theta-\beta)][n_2^2 \sin^2(\theta-\beta)-1]^{1/2}} d\varphi d\psi, \tag{10}
\]

\[
\psi_{\text{in}} = \arcsin \left[ \frac{(r-h)n_2}{r \sin \varphi_{\text{in}}} \right], \tag{11}
\]

and

\[
\psi_{\text{out}} = \arcsin \left[ \frac{(r-h)n_2}{r \sin \varphi_{\text{out}}} \right]. \tag{12}
\]

In this work, the sensing region of the probes had a double-pass, double-tapered structure; thus, the sensing region length and decay coefficient were \( 2L \) and \( 2\xi \), respectively. Hence, by using Eqs. (1), (3), (4), and (9), the effective light intensity transmitted through the fiber sensing region in which the evanescent waves decayed could be written as

\[
I_{\text{out}} = I_{\text{in}} \exp \left\{ -\frac{2\delta \lambda n_1 (K'_{(R,L,\beta,\epsilon,\delta,\Delta)\text{inner}} + K'_{(R,L,\beta,\epsilon,\delta,\Delta)\text{outer}})}{r(n_2^2-n_1^2)} \times 2L \right\}. \tag{13}
\]

Furthermore, Philip-Chandy et al. [28] discovered that the refractive indices of biofilms composed of mixed bacteria are between 1.330 and 1.355 when the biofilm thickness is between 0 mm and 2 mm; Bakke et al. [9] and Sandt et al. [29] also confirmed that the refractive indices of biofilms are close to that of water (1.333). However, the refractive index of fiber cladding is about 1.450. Thus, the refractive index of the biofilm \( n \) was less than \( n_2 \) in this study. Equation (13) can be further simplified by applying a Taylor expansion and using only the first
two terms of the Taylor series when the refractive index of the surrounding medium is smaller
than that of the fiber core, i.e., \((n/n_c) < 1\). Then, Eq. (13) can be expressed as

\[
I_{out} = I'_0 \eta_1 \exp(-\eta_1 n^2),
\]  
(14)

where

\[
\eta_1 = \exp\left[ \frac{4(\alpha_b + \alpha_l) \lambda L (K_{R,L,\beta,\lambda}^{\text{inner}} + K_{R,L,\beta,\lambda}^{\text{outer}})}{n c} \right]
\]  
(15)

and

\[
\eta_2 = \frac{4(\alpha_b + \alpha_l) \lambda L (K_{R,L,\beta,\lambda}^{\text{inner}} + K_{R,L,\beta,\lambda}^{\text{outer}})}{n c}.
\]  
(16)

Here, \(\alpha_b\) and \(\alpha_l\) are the bulk decay coefficients of the microbial cells and the liquid mixture in the biofilm, respectively. As Eq. (14) shows, the effective value of \(I_{out}\) is a function of \(\alpha\) and \(n\) when \(R, L, \beta, r,\) and \(\delta/\Delta\) are constant. However, as it is well known that biofilms consist of liquids and microbial cells embedded within a matrix of EPSs, for the Sen. probe, the refractive index \(n\) was corrected as follows:

\[
n^2 = V_b n_b + V_l n_l^2,
\]  
(17)

where \(V_b\) and \(n_b\) are the volume of microbial cells on the surface of the sensing region of the Sen. probe and the refractive index of the microbial cells in the biofilm, respectively; \(V_l\) and \(n_l\) are the volume of liquids in the biofilm and the refractive index of the liquid mixture in the biofilm, respectively. Thus, by using Eqs. (2), (14), and (17), the effective output light intensity of the Sen. probe can be further expressed as follows:

\[
I_{out,s} = K_s I_{in} \eta_1 \exp\left[ -\eta_1 (V_b n_b^2 + V_l n_l^2) \right],
\]  
(18)

where \(K_s\) is a function of \(\delta_s\) and \(\delta_s/\Delta_s\), and the subscript “s” on \(I, K,\) and \(\eta\) denotes the Sen. probe.

For the Ref. probe, the effective light intensity transmitted through the PSHF-coated sensing region (which could separate the liquids from the microbial cells) in which the evanescent waves decay can be written as

\[
I_{out,f} = K_f I_{in} \eta_2 \exp(-\eta_2 V_l n_l^2),
\]  
(19)

where \(K_f\) is a function of \(\delta_f\) and \(\delta_f/\Delta_f\), and the subscript “f” on \(I, K,\) and \(\eta\) denotes the Ref. probe.

According to Eqs. (12) and (13), when the parameters (i.e., \(\delta/\Delta, R, \beta, r,\) and \(\alpha\)) of the Ref. probe are approximately equal to those of the Sen. probe, the output signal of the sensor can be expressed as

\[
K = \frac{I_{out,f}}{I_{out,s}} = \frac{K_f}{K_s} \exp\left( \eta_2 V_l n_l^2 \right).
\]  
(20)

As Eq. (20) shows, the output signal \(K\) is a function of \(V_b\) and \(n_b^2\) when \(\delta/\Delta, R, \beta, r,\) and \(\alpha\) are constant, and \(K\) is not affected by changes in the liquid-phase composition or concentration. Furthermore, it is well known that the thickness of a biofilm has one-to-one correspondences with its refractive index and volume [8,9,24,29]; in particular, the biofilm volume \(V_b\), on the surface of the sensing region of the Sen. probe can also be expressed as \(V_b = LW x_b\), where \(W\) and

\(x_b\) are...
$x_b$ denote the sensing region diameter and biofilm thickness, respectively. Thus Eq. (20) can be expressed as,

$$K = \frac{I_{\text{out}, f}}{I_{\text{out}, s}} = \frac{K_f}{K_s} \exp \left( \eta_{\text{s}, s} LWx_b n_s^2 \right).$$  

(21)

Furthermore, Eq. (21) can also be expressed as a Taylor expansion as follows:

$$K = \frac{K_f}{K_s} \left[ 1 + \eta_{\text{s}, s} LWx_b n_s^2 + \frac{1}{2} \eta_{\text{s}, s}^2 (LWx_b n_s^2)^2 + \cdots \right].$$

(22)

It can be seen that, in Eqs. (21) and (22), the sensor signal parameter $K$ has a one-to-one correspondence with the biofilm thickness. Hence, the biofilm thickness can be determined by using the parameter $K$.

3. Results and discussion

3.1 Sensor performance parameters

The sensitivity, accuracy, and measurement range of the sensors depended on the attenuation of the evanescent waves in the biofilm. Hence, before monitoring the biofilm growth, we first examined the probes’ performance parameters, including their optical transmission characteristics, temperature responses, and sensitivities. For the PSHS-coated Ref. probe, the response time was also checked using glucose solutions.

Spectral transmission characteristics of probes. To obtain Sen. and Ref. probes with the same light transmission properties, we performed an optical transmission test. In the experiment, both ends of the probes were first polished and attached to standard SMA905 connectors. Then, the prepared fiber probes were placed in the channels of the PDMS membrane, and the BPBR was wrapped in opaque aluminum film and filled with distilled water at 30 °C. The transmitted light intensities were measured by employing an optical spectrometer (QE65000, Oceanoptics, USA), and the results are shown in Fig. 4(a). The total spectral scanning time was 2 s for each of the trials.

Temperature responses of probes. To investigate the effects of temperature on the transmitted light intensities of the Sen. and Ref. probes, we first investigated the relative changes in the transmitted light intensities (RCTLI) of the prepared probes with varying distilled water temperature. In the experiment, the water was heated from 25 °C to 40 °C at a fixed rate of 1 °C/min using a thermostatic bath to match the PSB strain culture temperature [8]. In Fig. 4(b), the parameter RCTLI was defined as $\text{RCTLI} = \left( I_T - I_1 \right) / I_1$, where $I_1$ and $I_T$ denote the transmitted light intensity of the investigated probe at 25 °C and at temperature $T$ (in the range from 25 °C to 40 °C), respectively; in this study, the $I_1$ values of the Sen. and Ref. probes were 18.41 nW and 18.44 nW, respectively.

Reference probe response time. As mentioned previously the physicochemical properties of the PSHF will affect the probe response time. To investigate the response characteristics in this study, an experiment was performed at 30 °C using various concentrations of a glucose solution (C$_6$H$_{12}$O$_6$·H$_2$O, 99%) as follows. Firstly, the reference probe was immersed in distilled water for about 5 min to thoroughly soak the PSHF. Secondly, glucose was intermittently added to the water until the glucose concentration reached 6 g/100 ml. Thereafter, the solution concentration was reduced by intermittently adding distilled water until it decreased to 1.5 g/100 ml; then, the probe was immediately immersed in the distilled water. Figure 4(c) shows the typical responses (transmitted light intensities) versus time that were captured by the Ref. probe (using a sampling interval of 1 s) with different glucose concentrations. In this work, the response time was defined as the time required for the probe output signal to stabilize when the probe was transferred from the 1.5 g/100 ml glucose solution to the distilled water.

Probe sensitivities. To investigate the response characteristics (linearities and sensitivities) of the prepared probes, we first installed the probes in the PDMS grooves, as shown in Fig. 2.
Then, the test solutions were prepared using the glucose and distilled water, and their concentrations ranged from 0 g/100 mL to 30 g/100 mL. In the experiment, the prepared solutions (30 °C) were pumped into the BPBR at a volumetric flow rate of 32 mL/h. The solution of each concentration remained in the BPBR for 5 min before being driven out of the BPBR for the next trial. The experimental results are shown in Fig. 4(d).

Figure 4(a) shows that the Sen. and Ref. probes exhibit the same spectral transmission characteristics in the range from 410 nm to 1100 nm. This similarity can be explained by the fact that the probes were prepared using the same fibers and the same etching conditions; thus, the etched probes exhibited the same parameters in the microscopic observations, such as surface roughness, fiber diameter, and taper angle. Furthermore, the porous membrane of the PSHF could effectively transmit water molecules; hence, the two probes experienced the same evanescent wave decays due to water molecule absorption and show the same spectral transmission characteristics.

In Fig. 4(b), the RCTLI of the Sen. probe increases only slightly with increasing temperature, while that of the Ref. probe increases significantly with increasing temperature, especially at temperatures above 35 °C, and reaches 0.006 at 40 °C. This RCTLI increase results from thermal effects on the refractive index of the PSHF and linear expansion with increasing temperature. Although the RCTLI of the Ref. probe increases with temperature, the largest percentage change in the light intensity transmitted by the Ref. probe is 0.6% at 40 °C. Thus, in this work, the effects of temperature on the probes’ output light intensities can be ignored between 25 °C and 40 °C.

In Fig. 4(c), the captured responses show that the transmitted light intensity varies significantly as a function of the glucose concentration with increasing time. The transmitted light intensity increases rapidly with increasing glucose concentration in initially low-concentration solutions, then increases more gradually, and eventually becomes relatively stable. Similarly, the transmitted light intensity decreases rapidly and then gradually becomes stable with the addition of water into high-concentration glucose solutions. These observations can be attributed to the fact that the concentration surrounding the etched fiber region is controlled by the diffusion rate of the glucose through the PSHF [30]. Figure 4(c) also shows that when the glucose concentration changes from 0 g/100 ml to 1.5 g/100 ml and from 1.5 g/100 ml to 0 g/100 ml the transmitted light intensity changes and Ref. probe response times are 0.646 nW and 0.703 nW and 157 s and 173 s, respectively. Thus, the PSHF-coated Ref. probe is effective and can respond quickly to liquid-phase concentration changes.

![Fig. 4. Performance parameters of prepared probes: (a) spectral transmission characteristics; (b) output light intensity variation versus temperature; (c) time response curve of Ref. probe; (d) output light intensity variation versus glucose concentration.](image-url)
In Fig. 4(d), the transmitted light intensities of the probes, $I_{out,1}$ and $I_{out,2}$, exhibit a linear relationship and rapidly increase with increasing glucose concentration, which can be explained as follows. Firstly, the difference between the refractive indices of the fiber and the glucose solution decreases linearly with increasing glucose solution concentration, decreasing the random scattering and refraction of light at pits on the fiber surface [31]. Secondly, in the glucose solution, the intensity of the light transmitted through the fiber is not affected by changes in the glucose solution concentration because no light (evanescent field) absorption occurs in the 410–1100 nm spectral region [8]; thus, $I_{out,1}$ and $I_{out,2}$ increase linearly with increasing glucose concentration. In addition, the transmitted light intensities of the Ref. and SEN probes maintained the same level because the probes had the same surface roughness and the same spectral transmission characteristics, as shown in Fig. 4(a).

3.2 Biofilm measurement

Biofilm growth on a support material begins with cell attachment and biofilm formation on the surface grooves and pores. Hence, the biofilm thickness, activity, growth rate, and structure are significantly affected by the groove depth (macroporosity) and the culture conditions because the macroporosity influences the contact area of the cells and the sorption–desorption properties [32,33] and because the culture conditions (i.e., steady-state and continuous-flow cultures) affect the fluid shear and substrate as well as the product transport. To examine and assess the performance of the prepared sensor, the biofilm growth on the PDMS with appropriate surface grooves (width and depth of 200 μm, which is close to the thickness of a typical PSB biofilm) was investigated using both continuous and intermittent flow cultures. Thereafter, the biofilm growth on the PDMS surface with deep grooves (width and depth of 200 μm and 550 μm, respectively) was investigated using a continuous flow culture; in this case, the depth was much greater than the thickness of a typical PSB biofilm. Furthermore, to calibrate the sensor, the biofilm growth was also monitored with an Olympus microscope.

3.2.1 Sensor response to biofilm using continuous flow culture

To test the sensor response to the biofilm development on the PDMS surface with grooves 200 μm in width and depth, the prepared probes were firstly fixed in the grooves; thereafter, biofilms were grown using a continuous flow of fresh medium and the method previously reported in [8], except that the bacteria suspension was recycled with a flow rate of 30 mL/h. The results of the probe microscope observations, sensor signals, fitting curve, simulated results, and environmental SEM images of the biofilm growth process are shown in Fig. 5. The sensor signals presented in Fig. 5(b) were calculated by comparing the transmitted light intensities of the Ref. and SEN probes by using Eq. (21). The simulated results were obtained by using Eq. (22), and the parameters used in the simulation are indicated in Table 1. The maximum refractive index step was set at 0.00006, and the parameters $\alpha_b$ and $\alpha_l$ were 100 mm$^{-1}$ and 20 mm$^{-1}$, respectively.

| Parameters | $L$ | $R$ | $b$ | $\theta$ | $h$ | $\alpha_b$ | $r$ |
|------------|-----|-----|-----|---------|-----|------------|-----|
| Value (Range) | 50 mm | 52.5 μm | 12.5 μm | $\pi$/4 | 25 μm | 60–100 mm$^{-1}$ | 6 mm |

| Parameters | $\alpha_b$ | $\lambda_x$ | $n_x (n_1)$ | $n_2$ | $\eta_{\max}$ | $n_b$ | $W$ |
|------------|------------|------------|-------------|------|--------------|------|------|
| Value (Range) | 10–25 mm$^{-1}$ | 850 nm | 1.4543 | 1.4500 | 1.4765 | 1.334–1.338 | 25 μm |

Figure 5(a) shows that $I_{out}$ decreases for each probe during the initial biofilm formation stage (in the biofilm growth process, the temperature of the BPBR was maintained between 27 °C and 33.5 °C; thus, the effects of temperature changes on the probe output signals were ignored). $I_{out}$ decreases because the substrate concentration decreased as it was consumed by the cells; however, $I_{out,s}$ also decreases due to the decrease in the evanescent wave intensity caused
by absorption by the biofilm cells. Thus, $I_{\text{out},s}$ decreases more than the output intensity of the Ref. probe, $I_{\text{out},r}$.

Thereafter, $I_{\text{out},s}$ in the continuous operation phase first increases, reaching its highest level at approximately 160 h, then decreases, and finally remains at a low level. This behavior can be attributed to the fact that the liquid-phase [substrate and product (formic, acetic, propionic, butyric, and valeric acids)] concentration increases due to the continuous supply of new synthetic medium, thus also increasing the metabolic activity of PSB cells [34]. Consequently, $I_{\text{out},s}$ increases with time until 160 h. However, as the biofilm porosity decreases and the EPS in the biofilm increases as shown in Fig. 5(c) (Samples E to F), the mass transfer resistance of the biofilm increases [32]. Thus, the substrate concentration at the fiber–biofilm interface decreased because of the increased mass transfer resistance, and the liquid product concentration also decreased at the interface due to the decreased biofilm cell activity. Furthermore, the evanescent wave attenuation was accelerated because the waves were absorbed by the denser accumulation of biofilm cells when the thickness was greater than 185 μm at a culture time of 160 h. As discussed above, $I_{\text{out},s}$ decreases when the biofilm thickness is greater than 185 μm [actually, the transmitted light intensity reaches its maximum at a biofilm thickness of 195 μm, as shown in Fig. 5(a), which can be mainly attributed to the microchannel in the PDMS with the hydrophilic surface]. The constant level of $I_{\text{out},s}$ can be explained as follows. The substrate and product concentrations reached equilibrium and the evanescent wave attenuation maintained a constant level because the bottom biofilm cells were not activated. The lack of bottom biofilm cell activation resulted from the fiber surface being coated thoroughly by the substrate; thus, the light from the LED was limited, inhibiting product generation.

Fig. 5. Probe output signals, biofilm thickness, sensor parameter $K_1$, and ESEM images of biofilm during biofilm development under continuous flow culture: (a) probe output signals and biofilm thickness versus culture time; (b) sensor signal, fitting curve, and simulated curve versus biofilm thickness; (c) ESEM images of biofilm at different culture times. In Fig. 5(a), $I_{\text{out},r}$ first rapidly increases and then decreases. Furthermore, compared with $I_{\text{out},s}$, $I_{\text{out},r}$ reaches a higher level, because the PSHF effectively separated the liquids from the PSB; thus, the Ref. probe measured the liquid concentration. The increase and the high level reached at 160 h are also mainly attributable to the increased liquid-phase concentration. The
$I_{out,r}$ decrease can be explained as follows. The biofilm thickness and cell density increased with increasing culture time, as shown in Fig. 5(c) (Samples A to F), increasing the mass transfer resistance and decreasing the biofilm activity and product production. Thus, the separated liquid-phase concentration in the probe sensing region decreased. Subsequently, $I_{out,r}$ maintained a constant level because the decreased substrate and increased product in the liquid phase of the BPBR reached equilibrium due to the light attenuation of the Ref. probe.

In Fig. 5(a), the biofilm growth also reveals adsorption, exponential, and stationary processes. In the adsorption process, the biofilm structure is quite loose, as shown in Fig. 5(c) (Sample A). In the exponential growth phase, the biofilm porosity gradually decreases, as shown in Fig. 5(c) (Samples B to D). Thereafter, the biofilm porosity further decreases, and the biofilm structure becomes denser due to product inhibition as well as substrate and light limitations, as shown in Fig. 5(c) (Samples E to F). Furthermore, based on the error bars shown in Fig. 5(a), the maximum relative error (MRE) of the biofilm thickness in the microscope observations was determined to be 9.5%.

In Fig. 5(b), the sensor signal increases as the biofilm thickness increases from 0 μm to 195 μm. To obtain the function relating the parameter $K_1$ to the thickness $x_1$, we performed multiple linear regression. The fitted function was determined to be $K_1 = 1E-05 x_1^2 + 0.0003 x_1 + 1.0136$ ($R^2 = 0.9939$). We found that the MRE between the fitted data and the sensor signals was 10.9% for biofilm thicknesses less than 195 μm. Furthermore, the MRE between the simulated results and the fitted data was 7.4% in the thickness range from 0 μm to 195 μm, because a single incidence angle $\theta$ and a single operational wavelength $\lambda$ were chosen in the simulation. This evidence supports the fact that the proposed sensor can be used to monitor biofilm development on a support material with appropriate grooves (width and depth both 200 μm) with a continuous flow culture.

3.2.2 Sensor response using intermittent flow culture

To examine the sensor response with an intermittent flow, the prepared bacterial suspension was recycled for 4 h with a flow rate of 30 mL/h. Afterwards, the BPBR was maintained in a steady state for 20 h. After that, 55 mL of medium was discharged and 55 mL of fresh nutrient medium was fed into the photobioreactor each day. The circulation flow rate and temperature of the medium were maintained at 30 mL/h and 30 °C, respectively. Figure 6(a) shows the sensor probe and microscope data obtained during online monitoring. Figure 6(b) depicts the sensor signal, fitting curve, and simulated results. The numerical simulation parameters are shown in Table 1; in particular, $\alpha_b$ and $\alpha_l$ were 90 mm$^{-1}$ and 20 mm$^{-1}$, respectively.

The light intensities transmitted by the probes vary with time identically in Figs. 6(a) and 5(a) during the first 40 h. However, between 40 h and 168 h and between 40 h and 185 h, the output signals of the Sen. and Ref. probes, respectively, fluctuate and continue to increase due to the adsorption and desorption processes.
to the intermittent supply of the fresh nutrient medium. To clarify the effect of the intermittent medium supply on the sensor output signals, we consider the following. First, the liquid-phase (substrate and product) concentration and its refractive index increased gradually as the fresh nutrient medium was supplied; thus, the light intensities transmitted by the probes increased. Then, the substrate concentration and its refractive index slightly decreased when the fresh nutrient medium stopped being supplied and was consumed by the bacteria; however, the product concentration increased due to the increase in biomass concentration, thereby indicating that the liquid-phase concentration was still greater than the concentration of the fresh nutrient medium supply before each change in medium. Hence, although the transmitted light intensity fluctuated, it still increased with culture time. At culture times above 228 h, i.e., for biofilm thicknesses near 170 μm, the output light intensity variations with culture time are again identical in Figs. 6(a) and 5(a). This correspondence can be attributed to the fact that the effect of the substrate concentration on the liquid-phase concentration in the fiber sensing region decreased gradually as the mass transfer resistance increased, due to the increase in biofilm thickness and decrease in biofilm porosity. Furthermore, compared with the Sen. probe, the transmitted light intensity maximum of the Ref. probe was delayed by 17 h, because some cells of the inter-layer biofilm continued to metabolize. Thus, the increased product concentration was difficult to transfer to the interface between the fiber and biofilm and was diffused into the bulk. Consequently, the product concentration of the solution further increased.

The biofilm development trend (see Fig. 6(a)) is also identical to the biofilm growth with a continuous fresh nutrient medium supply in Fig. 5(a). However, the thickness (172 μm) of the biofilm growth with an intermittent fresh nutrient medium supply is thinner than that of the film with a continuous fresh nutrient medium supply. The main reason for this difference is that, although an intermittent fresh nutrient medium supply, i.e., a closed cycle, can prevent the loss of activated PSB cells inside the circulated liquid solution, many cells will remain in the substrate limit state for a long time, thereby leading to low cell activity, a low growth rate, and high EPS content.

Again, using Eq. (21), the quantitative relationship between the sensor signal and biofilm thickness was obtained, and the results are shown in Fig. 6(b). This relationship was also found to be identical to that in the biofilm with a continuous fresh nutrient medium culture, which is shown in Fig. 5(b). The fitted function can be expressed as \( K_2 = 1E-05 \cdot x^2 + 0.0009 \cdot x + 0.9947 \) \( (R^2 = 0.9952) \). We found that the MREs between the fitted data and sensor signals and between the simulated results and fitted data were 9.8% and 13.4%, respectively, at thicknesses of 0–175 μm. Furthermore, by comparing the results calculated using the fitted functions, i.e., \( K_1 \) and \( K_2 \), the MRE between \( K_1 \) and \( K_2 \) was found to be about 6.3% at thicknesses of 0–195 μm. Therefore, the designed fiber sensor can accurately monitor biofilm growth in the thickness range from 0 μm to 195 μm (using PDMS with appropriate 200-μm-deep grooves) when a synthetic medium is supplied either continuously or intermittently to the photobioreactor.

### 3.2.3 Sensor’s detection limits

To examine the upper measurement limit of the prepared sensor, the groove depth in the support material (PDMS) was deliberately increased to 550 μm, which is much greater than the normal PSB biofilm thickness of about 180 μm [12,13]. In the experiments, the biofilms were grown with a continuous flow of fresh medium and were monitored using the prepared sensor. In addition, mature biofilms were sliced into four layers, from the bottom to the top of the biofilm, with layer thicknesses of 0–120 μm (Sample G), 120–240 μm (Sample H), 240–360 μm (Sample I), and 480–548 μm (Sample J); the active biomass in each sliced biofilm was examined to confirm the effectiveness of the measurement. Figure 7(a) shows the sensor probe and microscope data obtained during online monitoring. Figure 7(b) presents the sensor signal, fitting curve, and simulated results. The simulation parameters are shown in Table 1; in
particular, $\alpha_b$ and $\alpha_l$ were $60 \text{ mm}^{-1}$ and $10 \text{ mm}^{-1}$, respectively. Figure 7(c) depicts the active biomass in each sliced biofilm (Samples G to J).

In Fig. 7(a), the light intensities transmitted by the probes and the biofilm growth observed using the microscope exhibit the same trends as in Fig. 5(a). The reasons for these similarities can be obtained from the explanations of Fig. 5(a). It is also evident that, between 0 h and 240 h, the biofilm growth rate on the PDMS surface with 550-μm-deep grooves is faster than that on the surface with 200-μm-deep grooves, as shown in Figs. 5(a) and 6(a). The main reason for this difference is that deeper grooves can more effectively trap bacteria and improve cell attachment. Furthermore, at culture times after 288 h, the biofilm growth rate decreases and then maintains a constant level of 548 μm. The reasons for this behavior are as follows: first, the mass transfer resistance and attenuation of light from the LED increased with increasing biofilm thickness, leading to significant cell inactivation and even death (in Fig. 7(c), slightly red regions indicate substantial bacteria inactivation or death); second, the top biofilm reached adsorption–desorption equilibrium.

In Fig. 7(b), the sensor signal increases with biofilm thickness up to 536 μm, which occurs at 320 h. The fitted function can be expressed as $K_3 = 2 \times 10^{-6} x^2 + 0.0009 x + 0.9953 \quad (R^2 = 0.9931)$. However, at thicknesses greater than 536 μm, $K_3$ (sensor signal) decreases rapidly with increasing biofilm thickness. This trend can be explained as follows. Thicker biofilms were more likely to be diffusion-limited with increasing biofilm thickness and culture time, thereby leading to the sharp decrease in active biomass from top to bottom that are shown in Fig. 7(c) (Samples G to J). In particular, almost all of the bacteria were inactive in Sample G, causing the refractive index and liquid concentration (substrate and product concentrations) to change only slightly with increasing biofilm thickness and culture time; hence, the light intensity transmitted by the Sen. probe only slightly decreases for this sample. Furthermore, the cells in the top layer (Sample J) between 360 μm and 548 μm show high activity because of sufficient energy and substrate as well as low product inhibition, indicating that the top biofilm cells used the substrate for hydrogen production. Hence, the substrate and liquid product concentrations further decreased, causing the light intensity transmitted by the Ref. probe to further decrease in the biofilm thickness range from 536 μm to 448 μm (time range from 320 h to 440 h), as shown in Fig. 7(a). Consequently, the sensor output signal decreases with increasing biofilm thickness.

**Fig. 7.** Probe output signals, biofilm thickness, sensor parameter $K_3$, and active biomass in biofilms during biofilm development on PDMS with groove depth of 500 μm: (a) probe output signals and biofilm thickness versus culture time; (b) sensor signal, fitting curve, and simulated curve versus biofilm thickness; (c) images of active biomass in sliced biofilm samples (AP-EP denotes biofilm adsorption and exponential phases, SP denotes biofilm stationary phase).
Based on Fig. 7(b), the MREs between the fitted data and the sensor signal and between the simulated results and the fitted data were found to be 7.5% and 14.9%, respectively, at biofilm thicknesses less than 536 μm. The biofilm growth also exhibits adsorption and exponential phases at thicknesses of 0–536 μm in Fig. 7(a). Therefore, the effective measurement range of the prepared sensor is from 0 μm to 536 μm. The upper sensor measurement limit determined in this work is greater than those reported previously [8,11], which can be explained as follows. The U-shaped, conical probes enhanced the effective evanescent field intensity $I_{ev}$ and penetration depth $D_p$ because the angle $\theta$ at the core–cladding interface in the straight fiber region decreased to $\Phi$ at the outer fiber–biofilm interface ($\Phi'$ at the inner fiber–biofilm interface) in the U-shaped region, as shown in Fig. 3 [8, 20]. As $I_{ev}$ and $D_p$ increased, the light penetration depth in the biofilm increased; hence, the sensor exhibited a large measurement range. These promising experimental findings further indicate that the proposed fiber-optic sensor can be used to monitor PSB biofilm growth accurately (at thicknesses of 0–195 μm) in BPBRs with continuous or intermittent supply of a synthetic medium. In addition, when the biofilm thickness is forced to increase by increasing the groove depth, the sensor can effectively respond to the biofilm growth from the adsorption phase to the exponential phase in the thickness range from 0 μm to 536 μm. These abilities are attributable to the elimination of any effect of the liquid (substrate and product) concentration on the biofilm thickness signal by using the Ref. probe.

4. Conclusions

A novel microstructured, U-shaped, fiber-optic sensor with a double-tapered structure was developed to monitor biofilm thickness in a BPBR, and a model to demonstrate the biofilm thickness measurement principle was established. A PSHS was prepared and coated on the Ref. probe to separate the liquid from the PSB cells. The proposed sensor was employed to investigate biofilm growth on a PDMS support material with an appropriate groove depth (200 μm) in a BPBR with continuous and intermittent supplies of a fresh nutrient medium. To determine the upper measurement limit of the sensor, the groove depth was increased to 550 μm, which is much greater than the thickness of a normal PSB biofilm. Furthermore, to explain the effectiveness of the measurement, we examined the biofilm structure and active biomass in the biofilm. The experimental results showed that the Sen. and Ref. probes were highly sensitive and that the Ref. probe responded rapidly to changes in the glucose solution concentration. The biofilm thickness measurement results obtained during biofilm development using continuous and intermittent supplies of the synthetic medium to the bioreactor agreed well (with an MRE of 6.3%) at biofilm thicknesses of 0–195 μm, corresponding to the lag phase to the stationary phase in the biofilm culture, when a PDMS groove depth of 200 μm was used. When the PDMS groove depth used for biofilm growth was much larger than the normal biofilm thickness, the sensor still effectively monitored the biofilm growth from the adsorption phase to the exponential phase in the thickness range from 0 μm to 536 μm, with an MRE of 7.5%. Thus, the designed microstructured sensor can effectively and accurately monitor PSB biofilm development in a bioreactor across a wide measurement range. Therefore, this biofilm measurement technology, which is also easy to install and nondestructive, has great potential for use in chemistry, biochemistry, life sciences, and environmental research applications.

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