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Dynamic thermal sensor for biofilm monitoring☆

D.F. Reyes-Romero[a,*,1], O. Behrmann[a,1], G. Dame[a], G.A. Urban[a,b]

[a] Laboratory for Sensors, Department of Microsystems Engineering (IMTEK), University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[b] Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg, Albertstrasse 19, 79104 Freiburg, Germany

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ABSTRACT

A novel sensor principle to continuously monitor biofilm formation by *Enterococcus faecalis* is presented. The sensor consists of a small heater and a temperature probe (thermistor); both are located on a thermally insulating, silicon nitride membrane. Biofilm is allowed to develop in M17-medium at 37 °C on the surface of the membrane. Electrical current applied to the heater produces a steady sinusoidal power signal at 40 Hz. The resulting temperature oscillations are captured by the temperature probe. Their amplitude and phase shift are influenced by the thickness and composition of the biofilm. Results show that the sensor system is able to monitor the evolution of biofilms for large time frames (hours). It also features very stable baseline measurements that outperform other types of sensor systems. A proof-of-concept by means of an antibiotics-growth-inhibition test is achieved.

1. Introduction

Biofilms consist of bacteria embedded inside a surface-attached extracellular matrix, composed mainly of polysaccharides and proteins. In contrast to the free floating (planktonic) form of growth, biofilms provide several key advantages to the bacterial colony. These include increased protection from antibiotic substances and accelerated transport of nutrients and signal molecules inside the biofilm [1]. It has been shown that bacteria enter this specific form of growth as a response to extracellular conditions [2]. To study biofilm formation under the influence of different extracellular conditions, two main methods have been established [2,3]:

1. Biofilm is allowed to form on the surface of glass rods inserted into a fermentor. The attached cells are subsequently removed from the glass rods by sonication and enumerated by the plate count method.
2. Biofilm is allowed to form on the bottom of transparent polystyrene microtiter plates. After removal of any planktonic cells, the remaining biofilm is stained with 0.1% safranin and its absorbance is measured at 490 nm.

Since both methods are labor intensive and do not allow for real-time monitoring of biofilms, several efforts have been made to develop MEMS (Micro-ElectroMechanical-Systems) that are able to do so. Real-time monitoring does not only allow for tracking of biofilm development, but also for observation of dynamic responses of biofilms to the action of chemical agents. MEMS that both are, or may be, employed for real-time monitoring of biofilm dynamics can be categorized by their respective mode of operation:

- **Impedimetric**: Biofilm is allowed to form on top of either uninsulated [4,5] or insulated [6,7] planar electrodes. The change in electrical impedance caused by the surface attachment of the cells is used as a measure for biofilm development. Impedimetric systems are comparatively easy to manufacture, but are often hampered by low sensitivity and/or baseline drift due to electrochemical processes at the electrode–electrolyte interface.
- **Calorimetric**: Biofilm is allowed to form on top of an array of thermopiles [8]. The output voltage of the array is used as a direct measure of the heat flow caused by the metabolism of the biofilm.
- **Surface Acoustic Wave (SAW)**: Biofilm is allowed to form on a substrate between two interdigitated transducers (IDTs) [9]. The additional mass loading from the biofilm causes a proportional shift in the resonance frequency of the SAW device. However, for example, Kim et al. [9] have not yet been able to report reproducible measurements. Variations in the manufacturing process and effects of the cleaning process (Oxygen plasma) between experiments seem to be the cause. Because of the low penetration depth, only early stages in biofilm formation that occur very close to the sensor surface can be monitored.

☆ This document is a collaborative effort.

* Corresponding author. Tel.: +49 7612037267; fax: +49 7612037262.
E-mail addresses: reyes@imtek.de, diego.reyes@imtek.uni-freiburg.de (D.F. Reyes-Romero), behrmann@imtek.de (O. Behrmann), dame@imtek.de (G. Dame), urban@imtek.de (G.A. Urban).

[1] These authors equally contributed to this paper.

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- **Time-invariant (DC) heat transfer**: Biofilm is allowed to form on top of a heating element and a temperature sensor [10]. The temperature sensor is used to detect the heat flow between the heater and itself. Due to differences in the thermal properties of the biofilm [11,12] and the surrounding medium, the amount of heat transferred between the heater and the sensing element can be related to biofilm thickness.

The aim of this publication is to expand on the field of real-time monitoring of biofilm dynamics by using harmonic (AC) heat transfer. This method allows for the measurement of both the thermal conductivity and heat capacity of fluids [13,14]. Sensors for this purpose are typically constructed using thin-film technology and consist of at least one heating element and one temperature sensor deposited on a thermally insulating membrane [13–17]. The heating element sets a harmonic heat signal which produces temperature oscillations that propagate through the medium until reaching the temperature sensor. By analyzing the amplitude and phase shift of the measured temperature oscillations at a single frequency, it is possible to determine the thermal properties of fluids [17].

Because of their fully passivated construction, AC heat transfer sensors are inherently immune to baseline drift caused by electrochemical processes. This property makes them an ideal choice for measurements in liquids of high ionic strength.

To monitor a biofilm, a nutrient medium is placed on top of the sensor surface. The heater generates oscillating heat at a predefined frequency while a biofilm develops on the sensor's surface. Amplitude and phase shift of the temperature oscillations sensed by the temperature sensor serve as measuring parameters. Both of the signals are related to the thickness and the thermal properties of the biofilm.

2. Sensor

The sensor chip is structured on a silicon wafer. A 0.7 μm LPCVD silicon nitride layer forms the upper surface of the membrane. Then, lithography, physical evaporation and lift-off processes are used to structure the thermistors (germanium), the heater (chromium), and the conducting lines and paths (Ti/Au/Ti). An additional 0.7 μm PECVD silicon nitride layer is deposited over the structures as a passivation layer on the lower side of the sensor. Silicon above the membrane is etched with reactive ion etching (RIE) followed by 30%-KOH wet etching. The resulting membrane thickness is about 1.4 μm. A detailed description of the sensor geometry and the fabrication process is documented in previous contributions [15,18].

Fig. 1 shows a close-up of the membrane. In this application, only the central thermistor is used as a temperature sensor for measuring the temperature oscillations caused by the heater. Thin film germanium thermistors exhibit a thermal coefficient of resistance (TCR) of about −2%/K and a resistance of about 900 kΩ at 25 °C [18]. The temperature resolution is limited by noise to 0.1 mK for a bandwidth of 1 kHz and the typical time response is 3 ms [19].

The heater has a width of 6 μm, a length of 1.6 mm and a thickness of 100 nm. Chromium was selected as a material because of its low TCR, which is just about 0.01%/K [20]. The total resistance of the heater is 2.97 kΩ.

3. Theory

The measurement principle is based on the change in the dynamic thermal behavior as the biofilm develops on the sensor's membrane. Hence, it relies on the differences between the thermal properties between the liquid medium and the growing biomass. Steady sinusoidal heat generation occurs inside the heater depicted

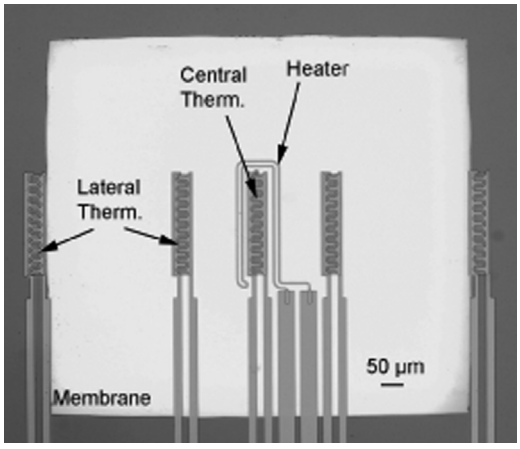

Fig. 1. View of the sensor's membrane. Only the central thermistor is used to measure the temperature oscillations. The average separation between the heater and the central thermistor is 6 μm.

in Fig. 2. This results in the creation of thermal waves that propagate away from the heater in all directions. As bacterial biofilm develops on the surface of the sensor, the effective thermal properties change, thus affecting the amplitude and the phase of the temperature waves (see Fig. 2). A temperature sensor, which lies on the same substrate as the heater, measures these changes in time.

The heat transfer through the culture medium is described by the heat diffusion equation [21]:

$$\nabla(k\nabla T(\boldsymbol{x},t)) + \dot{Q}(\boldsymbol{x},t) = \rho c_p \frac{\partial T(t)}{\partial t}, \tag{1}$$

where t is the time, T the temperature, $\dot{Q}(\boldsymbol{x},t)$ is the volumetric heat generation, k, ρ and c_p are the thermal conductivity, density and specific heat capacity of the medium, respectively.

A sinusoidal voltage of frequency $f/2$ is applied to the heater. Hence, the volumetric heat generation has a sinusoidal (AC) component of magnitude $|\Delta\dot{Q}|$ at a frequency f plus a DC-offset ($\dot{Q}_0$) as shown by

$$\dot{Q}(\boldsymbol{x},t) = \begin{cases} \dot{Q}_0 - |\Delta\dot{Q}|\cos(2\pi f t), & \text{in the heater} \\ 0, & \text{the rest} \end{cases}, \tag{2}$$

where $\dot{Q}_0 = |\Delta\dot{Q}|$.

Under steady state, the DC component of Eq. (2) sets a steady temperature distribution $T_0(\boldsymbol{x})$, which is obtained by reducing the right term of Eq. (1) to zero. In addition, the AC component of

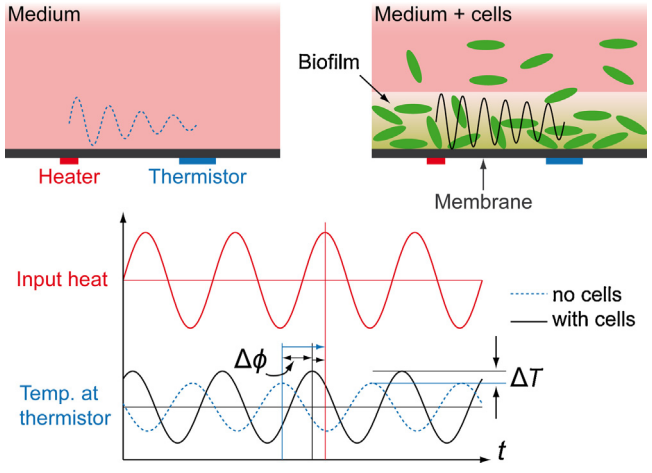

Fig. 2. As the biofilm forms, the amplitude and phase of the output signal changes.