

# Photoacoustic absorption spectra of biofilms

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Biofilms are aggregates of microorganisms which occur at the interfaces of natural and engineered aqueous systems. In this article, the use of photoacoustic spectroscopy (PAS) as a new technique for online and *in situ* monitoring of biofilms is demonstrated. Former studies revealed that PAS allows the monitoring of growth, detachment, and thickness of biofilms. Here, first photoacoustic absorption spectra of biofilms in the visible and near infrared range and corresponding depth-resolved measurements are presented. © 2003 American Institute of Physics.

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## I. INTRODUCTION

Biofilms are aggregates of microorganisms which occur at aqueous interfaces. Biofilms consist of microbial cells which are embedded in a matrix of extracellular polymer substances (EPS). Biofilms attached to solid surfaces can be found in natural and engineered water systems. The unwanted deposition of biofilms in technical processes is termed biofouling. Biofouling reduces the water quality and increases the frictional resistance in tubes. In wastewater treatment plants, biofilms are used for removal of organic and inorganic pollutants.<sup>1</sup>

For improvement of antifouling strategies and for process optimization in wastewater treatments plants, an online monitoring of biofilms is needed. Parameters as growth, detachment, thickness, and chemical composition of the biofilm have to be monitored nondestructively. In this article, the use of photoacoustic spectroscopy for biofilm monitoring is presented.

Pulsed photoacoustic spectroscopy is based on the absorption of electromagnetic radiation inside a sample where nonradiative relaxation processes convert the absorbed energy into heat. Due to the thermal expansion of the medium, a shockwave is generated which can be detected by piezoelectric transducers. The amplitude of the detected signal is proportional to the optical absorption coefficient of the sample.<sup>2</sup>

The time delay between the laser pulse and the arrival of the pressure wave at the sample surface is<sup>3</sup>

$$t = z/c, \quad (1)$$

where  $z$  is the distance between the sample surface and the absorbing layer inside the sample. Thus the time-resolved recording of laser-induced shockwaves allows the depth-resolved investigation of layered samples.

In this study, a setup for the indirect detection of laser induced pressure waves was used, i.e., excitation and detection of the pressure wave are performed from the same side of the sample.<sup>3</sup> The sensor heads were optimized for the

investigation of aqueous samples, especially hydrogels and biofilms.<sup>4</sup> Experiments with biofilm models consisting of agar-agar hydrogels<sup>5</sup> and first experiments with a biofilm showed the potential of PAS in the field of biofilm monitoring.<sup>6</sup> Growth, detachment, and thickness of biofilms were investigated by photoacoustic measurements.<sup>7,8</sup> In this article, first photoacoustic absorption spectra of biofilms in the visible and near infrared range are presented.

## II. EXPERIMENTAL SECTION

### A. Photoacoustic sensor system

A tunable, pulsed laser system was used for excitation of photoacoustic signals. A frequency-tripled ( $\lambda = 355$  nm) Nd:YAG laser (Surelite I-10, Continuum, Santa Clara, CA) with a repetition rate of 10 Hz pumped an optical parametric oscillator (OPO, Panther, Continuum, Santa Clara, CA). The OPO generates laser pulses with durations of a few nanoseconds. The wavelength of the emitted radiation can be tuned between 410 and 2550 nm. The laser pulses were guided via optical fibers (550  $\mu\text{m}$  diameter, HCG-MO550T-10, Laser Components, Santa Rosa, CA) to the photoacoustic sensor heads.

The available pulse energy at the distal end of the fiber was a function of the wavelength. The laser pulse energy was 0.4 mJ at 410 nm, 0.3 mJ at 420 nm, and 1 mJ at 430 nm. For higher wavelengths in the visible range, the laser pulse energy decreased with increasing wavelength and reached 80  $\mu\text{J}$  at 690 nm. In the near infrared, the laser pulse energy varied between 0.5 and 0.2 mJ in the wavelength range from 910 to 1190 nm. At 1580 and 2240 nm, the laser pulse energy was 0.2 and 0.1 mJ, respectively. All measured photoacoustic signals presented in this article were normalized to a laser pulse energy of 1 mJ and a temperature of 25 °C.

A photoacoustic sensor head consisted of a 25  $\mu\text{m}$  thick piezoelectric poly(vinylidene) fluoride film, which was coupled to a transparent prism by a conductive epoxy (Fig. 1). The piezoelectric detector was circular with a diameter of 5 mm, allowing a representative area of 20 mm<sup>2</sup>. The collimated laser light irradiates the biofilm growing directly on the surface of the prism. The detected signal was fed into a

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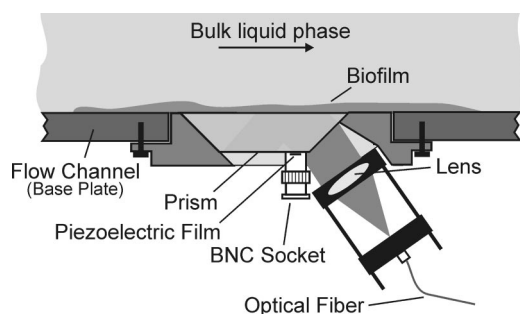


FIG. 1. Photoacoustic sensor head for biofilm monitoring.

preamplifier (HCA-100 M-50k-C current amplifier, Femto-Messtechnik, Berlin, Germany) and recorded by a four channel digital storage oscilloscope (TDS 540, Tektronix, Beaverton, OR).

In order to achieve absorption spectra, the OPO wavelength was tuned within the visible and the near infrared spectral range in steps of 10 and 20 nm, respectively. At each wavelength, three repeated photoacoustic measurements were performed. Every measurement was averaged over 100 laser pulses.

### B. Characterization of the sensor system

The sensor head used in this study was calibrated with well-defined dye solutions. The absorption coefficient was determined by UV-Vis spectrometry. The detection limit of the absorption coefficient was approximately  $0.02 \text{ cm}^{-1}$  and the relative standard deviation of repeated measurements was  $\leq 5\%$ .<sup>7</sup> The depth resolution was  $\pm 10 \mu\text{m}$ .<sup>5</sup> By use of the calibration function, all measured photoacoustic signal amplitudes presented in this article were converted into the corresponding absorption coefficients.

In order to examine photoacoustic measurements at variable wavelength, the photoacoustic spectrum of an aqueous solution containing  $100 \text{ mg L}^{-1}$  of the red textile dye "Sirius Lichtrot" (Hoechst, Frankfurt, Germany) was determined and compared with the absorption spectrum obtained with a UV-Vis spectrograph (Beckman, Fullerton, CA). For UV-Vis measurements, the sample was diluted by a factor of 100.

### C. Biofilm formation

A mixture of microorganisms taken from an aerobic sequencing batch reactor was grown in a 18 L tube reactor. The reactor contained a nutrient solution and was aerated with compressed air. To generate biofilms on solid surfaces, the content of the tube reactor was pumped through a flow channel by a peristaltic pump (Ecoline VC-280, Ismatec, Wertheim-Mondfeld, Germany) with a volume flow of  $250 \text{ mL min}^{-1}$ . In the middle of the 260 mm long flow channel a photoacoustic sensor head was integrated in the base plate of the channel. After 28 h, the flow channel was separated from the tube reactor to stop the adsorption of biofilm aggregates to the sensor surface. The biofilm was fed with 1 L of the nutrient solution and photoacoustic measurements were performed. A more detailed description of the experimental setup for generation and investigation of biofilms can be found in Ref. 7.

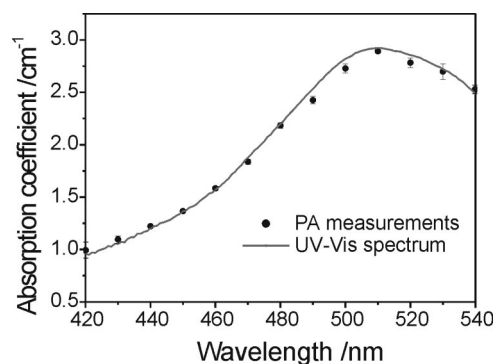


FIG. 2. Photoacoustic measurements of a red textile dye in the wavelength range of 420–540 nm. For comparison, the corresponding UV-Vis spectrum is shown.

## III. RESULTS AND DISCUSSION

### A. Characterization of the sensor system

Figure 2 exhibits photoacoustic signal amplitudes of the red textile dye "Sirius Lichtrot" measured in the wavelength range of 420–540 nm. For comparison, the corresponding UV-Vis spectrum is shown. The photoacoustic measurements are in good agreement with the UV-Vis spectrum of the sample. In this way, the determination of absorption spectra by the described photoacoustic technique could be verified.

Due to the mechanism of signal generation, photoacoustic spectroscopy allows the measurement of absorption spectra even of highly concentrated, optical opaque, and strongly scattering samples. Another advantage of photoacoustic spectroscopy over UV-Vis spectroscopy is the possibility of depth-resolved measurements.

### B. Photoacoustic absorption spectra of biofilms

In Fig. 3, the photoacoustic absorption spectrum in the visible range of a biofilm associated with the sensor surface is shown. The spectrum is a superposition of the absorption spectra of various pigments inside the microbial cells and the EPS matrix. The absorption maximum in the visible range is at 430 nm. For higher wavelengths, the absorption coefficient decreases with increasing wavelength.

By photoacoustic measurements using an OPO for excitation, even absorption spectra in the near infrared range (NIR) can be obtained. In Fig. 4, the spectrum of the biofilm

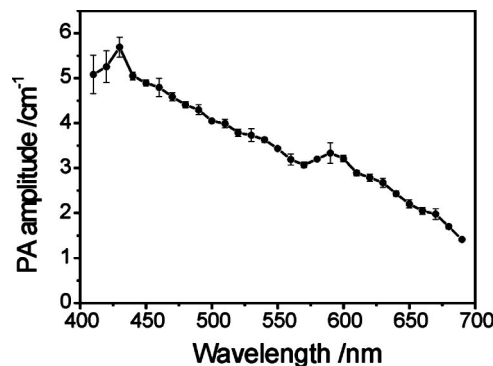


FIG. 3. Photoacoustic absorption spectrum of a biofilm in the visible spectral range.

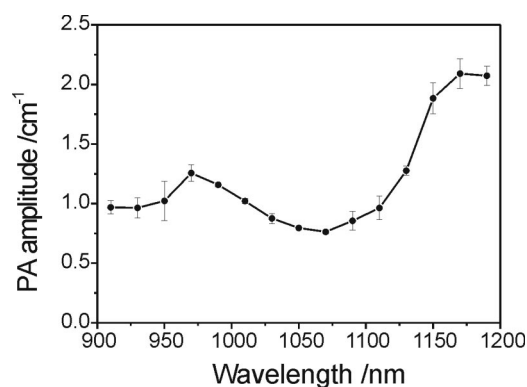


FIG. 4. Photoacoustic absorption spectrum of a biofilm in the NIR range.

from 910 up to 1190 nm is exhibited. Absorption bands of water at 970 and 1190 nm can be identified in the spectrum. Water is the main component of the bulk liquid phase and the biofilm itself.

### C. Depth-resolved measurements

As discussed in the Introduction, time-resolved recording of photoacoustic signal profiles allows depth-resolved investigation of inhomogeneous samples. The time scale can be converted into the corresponding depth scale by multiplying the time by the acoustic velocity of the sample. Biofilm and bulk liquid consist predominantly of water. Thus for the biofilm system the acoustic velocity of water  $c = 1.5 \text{ km s}^{-1}$  can be assumed.

In this way, depth-resolved measurements at three different wavelengths were performed (Fig. 5). At 440, 1580, and 2240 nm absorption bands of different components of the biofilm system can be observed. At 440 nm the incident light is absorbed by pigments, 1580 nm and 2240 nm are part

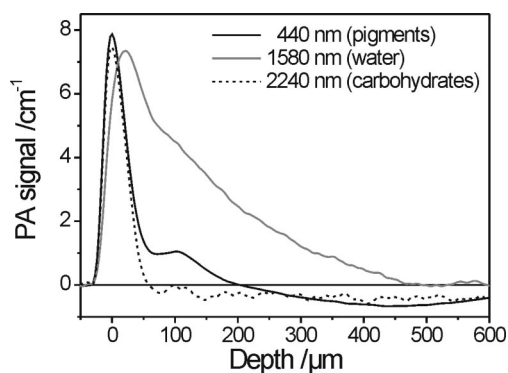


FIG. 5. Depth-resolved measurements of a biofilm at 440, 1580, and 2240 nm.

of absorption bands of water and carbohydrates, respectively. Pigments and carbohydrates are components of the biofilm. The concentration of these components in the bulk liquid could be neglected. In contrast, water is the main component of both biofilm and bulk liquid.

Due to the different distributions of the investigated compounds, the corresponding photoacoustic signal profiles have different shapes. The measurements of biofilm components leads to relatively narrow signals. Former investigations showed that the thickness of the biofilm can be estimated by determining the distance between maximum and minimum of the photoacoustic signal in the visible spectral range.<sup>7</sup> In this case, the distance between maximum and minimum corresponds to a biofilm thickness of  $450 \mu\text{m}$ . The photoacoustic measurement in the water absorption range at 1580 nm leads to a wider signal profile. This is due to the fact that water is a component of both the biofilm and the bulk liquid phase.

### IV. CONCLUSIONS

Photoacoustic spectroscopy (PAS) allows nondestructive investigation of biological samples. In this article, the use of PAS for monitoring of different major biofilm components is presented. By use of a tunable OPO system for excitation, wavelength dependent measurements could be performed. The determination of absorption spectra by photoacoustic spectroscopy could be validated by the measurement of a reference dye and comparison with the corresponding UV-Vis spectrum.

By PAS, absorption spectra in the visible and NIR range of a biofilm associated with the sensor surface could be determined. Via depth-resolved measurements, different distributions of various components of the biofilm system could be elucidated.

In further studies, specific staining reagents will be used to distinguish, for example, between living and dead cells. Stained cells and EPS components will be detected by photoacoustic measurements at specific wavelengths in a depth-resolved fashion.

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