

Biofilm monitoring by photoacoustic spectroscopy

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Abstract The use of photoacoustic spectroscopy (PAS) as a new biofilm monitoring technique is presented. Growth and detachment of biofilms at three different positions inside a flow channel were monitored by photoacoustic measurements in the visible spectral range ($\lambda = 532$ nm). The experimental approach allows the investigation of the influence of various process parameters (e.g. pH or flow conditions) on growth and detachment of biofilms. In addition, the distribution of the attached biomass can be monitored by depth-resolved photoacoustic measurements.

Keywords Biofilm; monitoring; photoacoustic spectroscopy

Introduction

Biofilms are aggregates of microorganisms, which occur at the interfaces of aqueous systems. Biofilms consist of water (85–95% wet weight), microbial cells, and extracellular polymer substances (EPS), such as polysaccharides, proteins, and other biopolymers. Biofilms which are attached to solid surfaces can be found in natural and engineered water systems. The unwanted growth of biofilms in technical processes reduces the water quality, increases the pressure differentials in membrane processes, and reduces the efficiency of heat exchangers. Despite these negative effects of biofilms, attached microorganisms are used also in beneficial applications, i.e. removal of organic pollutants in wastewater treatment plants (Wilderer and Characklis, 1989).

In the context of biofilm formation in technical processes, parameters such as growth, detachment, thickness, and chemical composition of the biofilm have to be monitored on-line by a nondestructive analytical technique. In this paper, the use of photoacoustic spectroscopy (PAS) for on-line monitoring of biofilms is presented.

Photoacoustic spectroscopy is based on the absorption of electromagnetic radiation inside a sample where non-radiative relaxation processes convert the absorbed energy into heat. Due to the thermal expansion of the medium, a pressure wave is generated which can be detected by microphones or piezoelectric transducers (Rosencwaig, 1980). The amplitude p of a photoacoustic signal generated by a laser pulse inside solid or liquid samples can be generally described by

$$p \propto \frac{\beta c^2}{C_p} E_0 \mu_a \quad (1)$$

where C_p is the heat capacity, β is the thermal expansion coefficient, c is the speed of sound in the medium under study, E_0 is the laser pulse energy, and μ_a is the absorption coefficient of the sample (Tam, 1986).

If a short laser pulse is used for excitation, a time-resolved recording of the photoacoustic signal allows a depth-resolved investigation of the light absorption inside the irradiated part of the sample (Karabutov *et al.*, 1995). The distance between an absorbing object inside the sample and the sample surface can be calculated as

$$z = c \cdot t, \quad (2)$$

where t is the time delay between the laser pulse and the arrival of the pressure wave at the sample surface. Thus, changes in the optical absorption properties of a sample can be investigated depth-resolved, if the sound velocity inside the sample is known.

In this study, a set-up for the indirect detection of laser induced pressure waves was used, i.e. excitation and detection of the pressure wave is performed from the same side of the sample (Karabutov *et al.*, 1995). The sensor heads were optimized for the investigation of aqueous samples, especially hydrogels and biofilms (Kopp and Niessner, 1999a). Experiments with biofilm models consisting of agar-agar hydrogels (Kopp and Niessner, 1999b) and first experiments with a biofilm showed the potential of PAS in the field of biofilm monitoring (Schmid *et al.*, 2001).

Growth, detachment, and thickness of biofilms can be monitored by photoacoustic measurements. The thickness can be obtained by measuring the distance between maximum and minimum of the depth-resolved photoacoustic signal profile. This method for thickness measurement was verified with biofilm models consisting of agar-agar. Thickness measurements of real biofilms by confocal laser scanning microscopy (CLSM) were in good agreement with the corresponding investigations by photoacoustic spectroscopy (Schmid *et al.*, 2002).

Methods

Photoacoustic sensor heads

A photoacoustic sensor head consisted of a 25 μm thick piezoelectric poly(vinylidene) fluoride film, which was coupled to a transparent prism by a conductive epoxy (Figure 1a). The piezoelectric detector was circular with a diameter of 5 mm, allowing a representative area of 20 mm^2 . The laser light was guided via optical fibers (HCG-MO550T-10, Laser Components, Santa Rosa, USA) with a diameter of 550 μm to the sensor head and collimated by a lens. The collimated laser light irradiates the biofilm growing directly on the surface of the prism. The detected signal was fed into a 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, USA). Both the preamplifier and the piezoelectric detector were shielded from electromagnetic interference. For investigation of biofilms at different positions, three photoacoustic sensor heads were integrated into a flow channel (Figure 1b).

The sensor heads were 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 cm^{-1} for all photoacoustic sensor heads used in this study and the relative standard deviation of repeated measurements was $\leq 5\%$ (Schmid *et al.*, 2002). The depth resolution was 10 μm (Kopp and Niessner, 1999a).

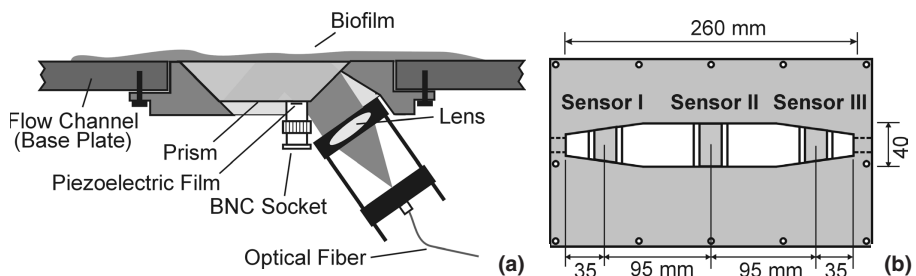


Figure 1 Photoacoustic sensor head (a) and flow channel with three integrated sensor heads (b)

Photoacoustic sensor system

A frequency doubled Nd:YAG laser (Surelite I-10, Continuum Corp.) with a repetition rate of 10 Hz and a pulse width of 5.4 ± 0.1 ns was used for excitation at $\lambda = 532$ nm. To improve the signal-to-noise ratio, the signal was averaged over 100 laser pulses. The synchronization of the digital storage oscilloscope with the laser pulse was performed with a master trigger. All devices in the sensor system were controlled by a in-house developed software (LABVIEW 5.1, National Instruments), which controlled flash lamp and shutter of the laser via a serial interface and the digital storage oscilloscope via the IEEE 488 bus.

Data analysis

As can be seen in Eq.(1), the amplitude of photoacoustic signals depends on the absorption coefficient, the laser pulse energy, and the physical properties of the sample. For biofilms consisting predominantly of water, the physical properties of water can be assumed. The temperature dependence of C_p , β , and c was corrected by use of literature data (Weast, 1983). The laser pulse energy was measured by a pyroelectric detector (Rj-7100, Laser Precision Corp.) and all recorded photoacoustic signals were normalized to a laser pulse energy of 1 mJ.

Aminosilanization of the sensor surfaces

To enhance the adhesion of cells to the prisms, the sensor surfaces were modified with an aminoalkyle silane. 1 mL of *N*-(2-aminoethyl)-3-aminopropyl-methyldimethoxy silane were dissolved in a mixture of 10 mL methanol and 10 mL water. The surfaces of the prisms were treated with this solution for 12 hours at room temperature.

Biofilm growth

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 consisting of 690 mg L⁻¹ sodium acetate, 60 mg L⁻¹ potassium dihydrogenphosphate, 252 mg L⁻¹ ammonium sulfate, 19 mg L⁻¹ potassium chloride, and 4 mg L⁻¹ yeast extract and was aerated with compressed air with a volume flow of 1 L min⁻¹.

To generate biofilms on the sensor surfaces, the content of the tube reactor was pumped through the flow channel by a peristaltic pump (Ecoline VC-280, Ismatec, Wertheim-Mondfeld, Germany) with a volume flow of 250 mL min⁻¹. The formation of biofilms was monitored by photoacoustic measurements.

Change of pH and biofilm detachment

After 28 hours, the flow channel was separated from the tube reactor and the biofilm was fed with 1 L of the nutrient solution described above. After 20 hours, 10 mL of acetic acid (99.8%) were added and the pH was shifted from 7.5 to 3.5. Changes of the attached biomass were monitored by photoacoustic measurements.

Results

The content of the tube reactor was pumped through the flow channel with a volume flow of 250 mL min⁻¹ and the growth of biofilms was monitored by photoacoustic measurements. Figure 2 shows the photoacoustic signal profiles of sensor heads I and II during the first 80 minutes of the experiment. The increase in signal intensity is due to the attachment of microbial cells and EPS molecules to the surface of the prisms. The biofilms on sensor heads II and III showed similar behavior. Therefore, only the signals of sensor heads I and II are presented in this paper.

According to Eq. (2), the time scale was converted into the corresponding depth scale.

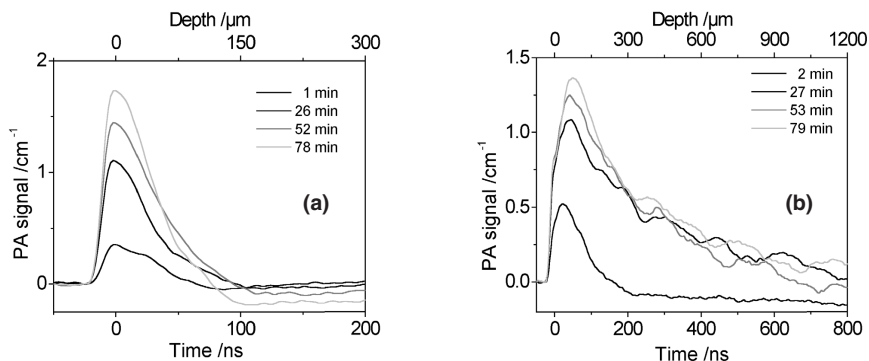


Figure 2 Photoacoustic signal profiles ($\lambda = 532$ nm) of biofilms growing on sensor heads I (a) and II (b)

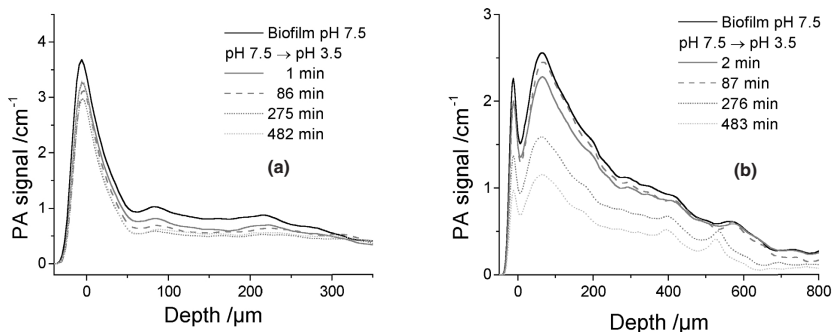


Figure 3 Biofilm detachment by pH shift from 7.5 to 3.5: photoacoustic signal profiles of sensor heads I (a) and II (b)

The conversion factor was the sound velocity of water $c = 1,500 \text{ ms}^{-1}$. The value $0 \text{ } \mu\text{m}$ corresponds to the surface of the prism.

Obviously, the distributions of the attached biomass on sensor heads I and II differ from each other. These differences are due to different flow conditions inside the flow channel. At the beginning of the experiment (Figure 2, “1 min” and “2 min”, respectively), biofilms with thickness $150 \text{ } \mu\text{m}$ and $300 \text{ } \mu\text{m}$ were generated. At later times, only the flow conditions on sensor heads II and III allowed the attachment of biofilm flocs and the formation of biofilms with thickness $> 1 \text{ mm}$.

Figure 3 shows the photoacoustic signal profiles of biofilms before (biofilm pH 7.5) and after addition of 10 mL acetic acid (99.8%) to 1 L nutrient solution. After the change of pH from 7.5 to 3.5, approximately 60% of the attached biomass on sensor head II was removed within 8 hours. The pH influences electrostatic interactions between EPS molecules and in this way, changes of the pH can influence the stability of biofilms.

The biofilm detachment on sensor head II is approximately uniform in the depth range $0\text{--}800 \text{ } \mu\text{m}$. In contrast to sensor head II, biofilm detachment on sensor I could be observed only from $50 \text{ } \mu\text{m}$ to higher layers. In this range, approximately 50% of the biomass were removed after addition of acetic acid. The signal of the base biofilm ($0 \text{ } \mu\text{m}$) varied only in the range of $\pm 10\%$. Obviously, different biofilm structures and flow conditions lead to different stabilities of the biofilms.

Conclusions

Photoacoustic spectroscopy (PAS) allows nondestructive investigation of biofilms. By photoacoustic absorption measurements in the visible spectral range ($\lambda = 532 \text{ nm}$), growth,

detachment, and thickness of biofilms which are associated with the sensor surface can be monitored depth-resolved. The described experimental method allows the investigation of the effects of various process parameters, e.g. pH or flow conditions. Current studies are devoted to investigations of efficiency of several anti-fouling strategies.

Further studies will utilize an optical parametric oscillator (OPO) which is pumped by a Nd:YAG laser. The OPO allows the generation of short laser pulses in the wavelength range from 410 nm to 2550 nm. With this system, photoacoustic absorption spectra of biofilms can be measured and biofilm components can be monitored in a more specific way due to their characteristic absorptions. Furthermore, additional CLSM measurements will be performed for a more extensive comparison between photoacoustic spectroscopy and an independent microscopic imaging technique.

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