A UV-LED Based Optical Fiber Biofilm Sensor: Design, Calibration, and Field Application

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An optical fiber-based biofilm sensor has been developed in order to dynamically detect biofilm formation of bacteria and unicellular microorganisms in their natural marine environment. Surfaces submerged in water get naturally rapidly covered by bacteria followed by multicellular eukaryotic micro- and macroorganisms forming a mature biofilm. Several optical, electrochemical and micromechanical sensors have been developed focusing on a wide range of biofilm characteristics. However, field sensors meet challenges which differ from those highly sophisticated laboratory instrumentations. Therefore we developed a sensor which allows us to study biofilm formation dynamics in-situ, non-destructively, online and selectively. The device is based on the detection of natural fluorescence utilizing the intrinsic amino acid tryptophan of microorganisms constituting the biofilm. Promising sensor head geometries were modeled and optimized in terms of spatial arrangement of the entire optical system and of the light emission and collection characteristics. The sensor head design is capable of detecting biofilms grown on a large surface of about 0.5 cm² (patent pending: Fischer, Friedrichs, Wahl; DE 102011101934.4). The intrinsic fluorescence originating from biofilms disposed on a UV transparent substrate is excited by a 280 nm UV LED. The emitted fluorescence is
collected and guided by 540 optical fibers to a photomultiplier tube operating in photon counting mode. Interference filters are utilized to spectrally separate the 350 nm fluorescence emission from background and scattered excitation light. Calibration measurements with a tryptophan dilution series show a linear correlation between fluorescence intensity and concentration. The sensor signal shows a dynamic range from nanomolar to millimolar tryptophan concentrations. Two marine bacteria strains have also been tested by analyzing the cell number and coverage area of the biofilm. The detection minimum of 4000 bacteria cells/cm$^2$ was estimated and reveals that the sensor is capable of studying biofilm establishment from the first attachment of cells up to complex structured biofilms. In our field experiments, biofilm formation dynamics has been continuously monitored by exposing the sensor to Baltic Sea water over a period of several weeks. The fluorescence intensity was measured hourly (10ms measurement time each) and subsamples were collected for a comparison of the biofilm sensor readout with fluorescence microscopy images. To quantify accumulated cell density the bacteria were stained by the fluorescent dye DAPI. Twenty random images of each stained biofilm were captured and analyzed by a program implemented in the software ImageJ. Additionally, the composition of the bacterial community has been analyzed by fluorescence in situ hybridization (FISH) using Cy3-labeled oligonucleotide probes. In summary, the biofilm sensor measurements clearly proved for the first time that straightforward continuous monitoring of biofilm formation in natural habitats is feasible. Moreover, the sensor holds potential for deep sea deployment and industrial applications.