Structural and Temporal Behaviour of Biofilms Investigated by FTIR - ATR Spectroscopy

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The temporal and physiological behaviour of bacteria forming biofilms on a surface was investigated by FT-IR ATR spectroscopy. Time dependent spectra could be attributed to changes in biofilm properties. H-D exchange experiments offered insights in structural changes of biofilms after chemical treatment with chlorine, used as a desinfectant.

INTRODUCTION

Most bacteria on earth live in an immobilized form, embedded in extracellular polymeric substances (EPS) and adhere to surfaces in soils, sediments and many other habitats which provide interfaces, water and nutrients. This is referred to as biofilms. Their metabolic activity can change significantly with physico-chemical properties of the supporting material and the micro environment, e.g., influencing the kinetics of corrosion processes. An economic property of biofilms is the binding of water; this has to be overcome when sludge has to be dewatered. In order to study biofilms, non-destructive methods are required, in which structure of the matrix and concentration gradients are maintained. As a non-destructive, on-line and in situ method, FTIR-ATR is used for monitoring bacterial adherence, biofilm formation and development directly at the water/solid interface (1). In a specially designed ATR-optical device, biofilm studies can be performed over a period of days. Sample and reference spectra were obtained in quasi-real time in the single-beam, sample reference (SBSR) technique (2). Structural investigations of biofilms were made using H-D exchange.

METHODS

Biofilms were grown on the internal reflection element (IRE) of an ATR spectrometer cell after inoculation of the surface with bacteria in a continous flow through system. As test strains, a *Pseudomonas aeruginosa* SG22 and mixed cultures from a potable water system were used. Nutrients were supplied continously in the desired concentration (0.7, 1.5, 2 mg/l Caso bouillon). Based on the theoretical considerations about the electric field vector in the ATR-plate, sample and reference were measured on a single crystal without interference. This results in ATR-spectra, where long-term measurements can be performed without water/water vapour compensation and instrument instability. H-D exchange was performed in the fully hydrated biofilm through the gas phase, with an H_2O/air and D_2O/N_2 mixture at constant relative hymidity, temperature and the pH-value, the latter as far as possible. Measurements were performed on a Bruker IFS 66 FT-IR spectrometer (Bruker Karlsruhe) and an MCT-detector.

RESULTS AND DISCUSSION

Physiological studies

The IR spectrum of biofilms shows specific bands of proteins, polysaccharides, phosphoryl compounds and other groups of molecules (3,4). Biofilms are complex microbial systems and respond to nutritional conditions. Physiological changes are also possible in response to degraded and/or sorbed substances (1). It is of interest to know if and how much the activity may change and which concomitant physiological alterations occur. The detection of physiological and temporal changes of bacterial cells within biofilms due to different nutrient concentrations could be improved by the introduction of the SBSR-technique. No water and water vapour compensation is necessary in long term measurements,

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which otherwise leads to unsatisfactory signal to noise ratios due to subtraction and further mathematical data treatment (e.g. derivatives) is limited.

Figure 1 gives a typical example of stacked spectra, representing biofilm growth. After only one hour the cells can be observed beginning to attach to the crystal surface. This is first indicated by the occurrence of the amide I and II bands. Within 24 hours a rapid increase in cell numbers on the surface takes place until a plateau phase is reached. The spectra demonstrate that the

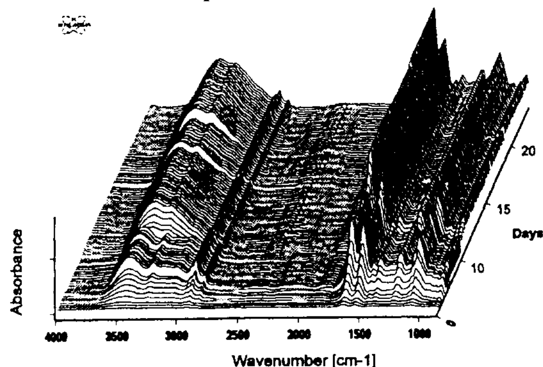

FIGURE 1. Stack plot of biofilm growth. ATR-FT-IR spectra aquired without any further data treatment (e.g.water subtraction, water vapour compensation)

intensity and composition of the bands between 1200 cm^{-1} and 900 cm^{-1} vary considerably during the measurement time of 120 hours according to the physiological response of the biofilm. These regions are mainly correlated with the formation of extracellular polymeric substances (EPS). This means that the physical and chemical properties of the EPS [5] may change more than expected during biofilm growth. Furthermore and with time, a decrease in EPS associated bands, a decrease in phosphate bands and differences in ratios and band shapes of amide I and II are observed. The decrease of the phospate bands is correlated by low cell activity. Changes in protein composition are manifested in morphological changes of the cells. They become smaller due to starvation.

H-D exchange in biofilms

It is well known that the spatial structure of biofilms change during growth. These changes were invesitgated by H-D exchange of the protons in the biofilm matrix, which consists significantly of bound water. Figure 2 shows the exchange kinetic of a mature biofilm.

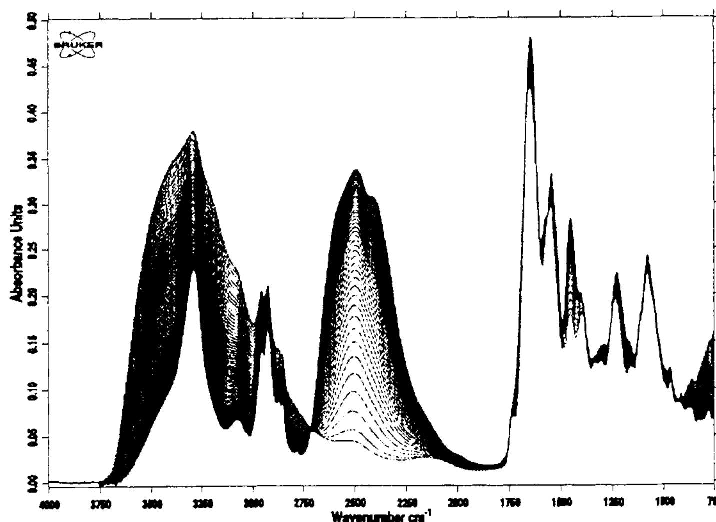

FIGURE 2. H-D exchange kinetic in a mature biofilm (5 days growth). Decrase in O-H (3400-3000 cm^{-1}) and increase in O-D bands (2700 -2000 cm^{-1})

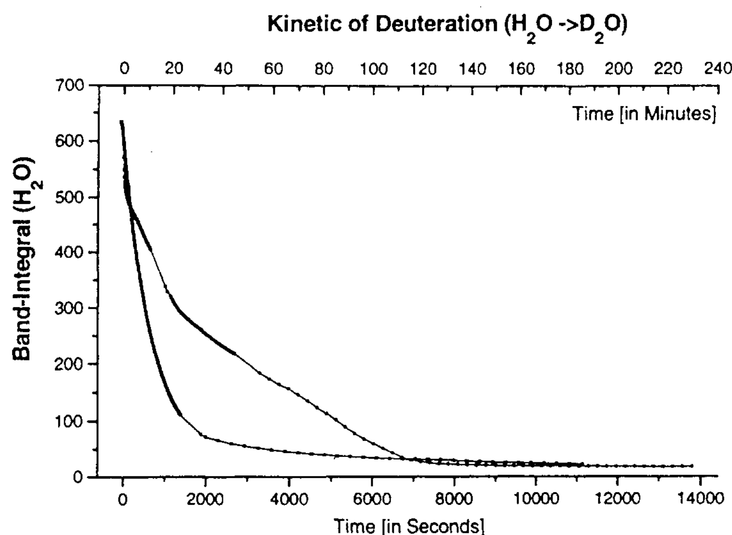

Figure.3. Exchange rates of biofilms treated with 6mg/l chlorine (below) and without (above).

The functional groups that are readily accessible are exchanged rapidly within the first 10 minutes of the deuteration process. The rate slows down between 15 and 30 minutes and finally falls off almost linearly. The exchange of the protons of interest is completed after 2 hours. Agents, which are suspected to alter the EPS matrix (6) show a significant change in the H-D exchange kinetics. Figure 3 shows the exchange rates of two biofilms, one treated with chlorine which is common in drinking water desinfection and without. The biofilms in drinking water, mixed culture biofilms, are grown under identical conditions for 2 days with 2 mg/l Caso bouillon.

It is obvious, that the exchange rate of the chlorinated biofilm is much faster, following an exponential decay. The untreated biofilm shows a multifunctional behaviour, with several underlying exchange processes. This suggests, that the chemical treatment of biofilms (e.g. chlorination) results in the destruction of coherence in the EPS matrix, as caused by the oxidative action of chlorine. The destruction of the EPS matrix again changes the physical and chemical properties within biofilms. This may result in different diffusion coefficients, in nutrient supply and in the mechanical resistance.

CONCLUSIONS

The complex matrix of biofilms was investigated by FT-IR / ATR spectroscopy biofilms in situ, on-line and non-destructively. The developement of a new ATR accessory enabled us to study the physiological behaviour of biofilms in long-term measurements, practically without limits of the spectrometer stability, water and water vapour interferences.

Spectral changes can be observed very sensitively and attributed to biochemical/mircobial reactions. This provides a tool for testing microorganisms attached to surfaces to chemicals used for cleaning, desinfection and in biomedical applications. Here, drug delivery and antibiotic testing is of interest.

The hydrogen/deuterium exchange in biofilms provides insight into the EPS matrix and hence in the spatial structure of biofilms. Different underlying exchange processes must be assumed which are still to be determined in further detail. Differences in the exchange rates can be quantified and attributed, resulting in a more mechanistic understanding of the biofilm matrix.

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