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1)Nature: Biofilms — matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces — represent a significant and incompletely understood mode of growth for bacteria. Biofilm formation appears early in the fossil record (∼3.25 billion years ago) and is common throughout a diverse range of organisms in both the Archaea and Bacteria lineages, including the 'living fossils' in the most deeply dividing branches of the phylogenetic tree. It is evident that biofilm formation is an ancient and integral component of the prokaryotic life cycle, and is a key factor for survival in diverse environments. Recent advances show that biofilms are structurally complex, dynamic systems with attributes of both primordial multicellular organisms and multifaceted ecosystems. Biofilm formation represents a protected mode of growth that allows cells to survive in hostile environments and also disperse to colonize new niches. The implications of these survival and propagative mechanisms in the context of both the natural environment and infectious diseases are discussed in this review.

B)Industries: Growth of biofilms in food processing environments leads to an increased opportunity for microbial contamination of the processed product. This increases the risk of reduced shelf life and disease transmission. Microorganisms within biofilms are protected from disinfectants (Frank and Koffi 1990, McCarthy 1992, Ronner and Wong 1993), increasing the likelihood of survival and subsequent contamination of food. EPS associated with biofilms that is not removed by cleaning provides attachment sites and nutrients for microorganisms newly arrived to the cleaned system (Hood and Zottola 1997). Wong (1998) reported that undesirable microorganisms such as Lactobacillus curvatus and Lactobacillus fermentum persisted on milk residues in cheese processing plants even after repeated cleaning, subsequently contaminating products. Reduction in the efficiency of heat transfer (Mittelman 1998) can occur if biofilms become sufficiently thick at locations such as plate heat exchangers. Some microorganisms in biofilms catalyze chemical and biological reactions causing corrosion of metal in pipelines and tanks.

C)Hospital: The application of confocal scanning laser microscopes (CSLM) to biofilm research radically altered our perception of biofilm structure and function .Before the use of CSLM, electron microscopy was the method of choice to examine microbial biofilms under high resolution. Unfortunately, sample preparation for electron microscopy results in dehydrated samples. Consequently, this approach provided a deceivingly simplistic view of biofilms, since the biofilm collapsed when water was removed. On the other hand, CSLM, which allows the visualization of fully hydrated samples, has revealed the elaborate three-dimensional structure of biofilms . CSLM has been used very effectively to monitor biofilm development in flow cells. Flow cells are small continuous-flow systems with a viewing port that allows direct observation of the biofilm without disrupting the community.

On medical devices: Low-energy surface acoustic waves generated from electrically activated piezo elements are shown to effectively prevent microbial biofilm formation on indwelling medical devices. The development of biofilms by four different bacteria and Candida species is prevented when such elastic waves with amplitudes in the nanometer range are applied. Acoustic-wave-activated Foley catheters have all their surfaces vibrating with longitudinal and transversal dispersion vectors homogeneously surrounding the catheter surfaces. The acoustic waves at the surface are repulsive to bacteria and interfere with the docking and attachment of planktonic microorganisms to solid surfaces that constitute the initial phases of microbial biofilm development. FimH-mediated adhesion of uropathogenic Escherichia coli to guinea pig erythrocytes was prevented at power densities below thresholds that activate bacterial force sensor mechanisms. Elevated power densities dramatically enhanced red blood cell aggregation.

**2)BIOFILM PREVENTION: CAN BIOFILM BE CONTROLLED?**
At present there is no valid method for complete biofilm prevention. A continuous chlorine level can help control biofilm, but it can also persist in chlorinated pools. In addition, once formed, it is very difficult to remove the biofilm and keep it away. The main prevention strategy is the**regular cleaning and disinfection**, to prevent bacteria from strongly sticking to surfaces. Most disinfectants are more effective in absence of organic compounds. In addition, temperature, pH, water hardness, chemical inhibitors, concentration and contact time generally affect their effectiveness.

Disinfection strategies should achieve the following outcomes:

- Destroy polysaccharide matrix and microorganisms left after cleaning
- Maintain biofilm-free all equipment, inhibiting intermittent biofilm formation
- Maintain or improve the overall quality of the water

Among the oxidizing agents, chlorine dioxide is known to be more effective and safe, when compared with chlorine:

However, to be effective, chlorine dioxide concentration should be at least 0.1 mg/L and should not exceed 0.8 mg/L (according to Environment Protection Agency), and 0.8 mg/L might not be enough to kill completely Legionella. Therefore, it may be necessary to use it at a higher concentration (maybe at 1–2 mg/L) and to provide alternative sources of potable water until Legionellae spp. are shown to be under control.

Bio-enzymatic cleaners, also known as “green chemicals”, or enzyme-based detergents, containing enzymes able to destroy protein-polysaccharide complexes, could contribute to biofilm degradation

3) **Sampling and fixation of biofilm.**Biofilm samples for cryosectioning and DNA extraction were taken on 28 February 2006 from the pilot plant trickling filter NTF2 and MBBR T1. Full-scale NTF samples were taken on 21 May 2003, as previously described. Snails, worms, and other animals were removed before the biofilm was retrieved. For DNA extraction, the biofilm was brushed off with a toothbrush and suspended in 1× phosphate-buffered saline (PBS) in 1.5-ml Eppendorf tubes. The biofilm suspensions were centrifuged for 3 min (5,000 × g). Pellets for DNA extraction were kept at −20°C until use. Samples for cryosectioning were fixed by submerging the plastic pieces in 4% paraformaldehyde for 8 h at 4°C, followed by rinsing twice with PBS before application of the cryosectioning protocol.

**Cryosectioning.**Biofilm cryosections were produced as previously described. After fixation, the plastic pieces were covered with a thick layer (several millimeters) of Tissue-Tek O.C.T. Compound (Sakura Finetek Europe B.V., The Netherlands) on the biofilm side, placed in a closed and parafilm-sealed petri dish, and incubated overnight at 4°C. Excess O.C.T. compound was removed from the back side of the plastic pieces, and a second layer of O.C.T. compound was added at the biofilm side to an approximate thickness of 2 mm. The embedded pieces were placed in a liquid nitrogen fume chamber until the O.C.T. compound was completely frozen. The plastic edge of such a piece was then gently bent, by using forceps, to detach the frozen block containing the biofilm. Subsequently, the detached biofilm blocks were again embedded in O.C.T. and placed in the liquid nitrogen fume chamber until frozen solid. The blocks were stored at −70°C until use. They were sectioned in 10-μm-thick vertical slices with a HM550 microtome cryostat (Microm International GmbH, Walldorf, Germany) at −20°C. The slices were collected on SuperFrost Plus Gold microscope slides (Menzel GmbH, Braunschweig, Germany). After dehydration in an ethanol series (50%, 80%, and 96% [vol/vol]), the microscope slides were stored at −20°C until use.

**Fluorescence in situ hybridization.**To ensure that all cells in the sliced biofilm would be fixed, the cryosectioned biofilm samples were fixed again directly on the microscope slides at room temperature for 20 min with 4% paraformaldehyde, followed by submersion in PBS for 30 min. FISH was performed at 46°C for 4 h. To facilitate FISH, a hydrophobic barrier frame was applied to the glass slides around the regions containing biofilm sections by using a Liquid Blocker Mini Pap Pen (Daido Sangyo, Tokyo, Japan). Probes specific for nitrifiers were hybridized together with the EUB338 probe mixture (labeled with a different fluorochrome) to biofilm samples. When probes with different hybridization stringency optima were applied to the same sample, several hybridizations were performed, beginning with the probe(s) requiring the most stringent conditions. The probe sequences and hybridization conditions are listed in Table S1 in the supplemental material.

For sequential FISH of the cryosectioned biofilms, probes specific for AOB and NOB were used in the first round of in situ hybridization and microscopy. While recording the images of these probe signals by confocal laser scanning microscopy, the field of view (FOV) position on the glass slide, indicated as x-y coordinates provided by the motorized microscope stage control unit, was recorded and later used for positioning during image acquisition of the bacterial reference area. After carefully removing the coverslip and rinsing the glass slide in double-distilled water, a second hybridization step was performed with the EUB338 probe mixture only (with 10% formamide in the hybridization buffer). New images containing the EUB probe signal were recorded at the same locations in the biofilm where images had already been taken after the first FISH step. Minor adjustments of confocal microscope settings were made to obtain optimal congruency between the micrographs of the nitrifying community and the bacterial biofilm reference. Corresponding images showing the same FOV were aligned and superimposed using Photoshop CS4 Extended (Adobe Systems, San Jose, CA, USA). Pairs of corresponding images were carefully compared to ensure that the shape and size of identical biomass objects had not been altered during the repeated hybridization and image acquisition procedures. In the rare cases where biofilm pieces had been lost or partly destroyed, the affected images were excluded from further analysis. All fluorescent probes and unlabeled competitors were obtained from Thermo Electron (Interactiva Division, Ulm, Germany) or MWG Biotech (Ebersberg, Germany). Fluorescent probes were 5′ labeled with the sulfoindocyanine dye indocarbocyanine (Cy3) or indodicarbocyanine (Cy5) or with fluorescein or Alexa 488 dye. In addition, probe Nse1472 was used with double labeling of oligonucleotide probes (DOPE) (5′ and 3′ labeled with Cy3) . All slides were mounted in Citifluor AF1 (Citifluor Ltd., London, United Kingdom) prior to microscopy.

**Microscopy and digital image analysis.**Images were collected using a Bio-Rad Radiance 2000 MP confocal laser scanning microscope (Bio-Rad, Hemel Hempstead, United Kingdom) equipped with a red diode laser (638 nm), a He/Ne laser (543 nm), and an argon laser (457, 476, 488, and 514 nm). Images for quantification were collected, using a Nikon Plan Fluor 40×/1.40 oil objective and the bundled software LaserSharp 2000, as 8-bit/pixel greyscale or 24-bit/pixel RGB images of 512 by 512 pixels (resolution, 1.65 pixels/μm). A Kalman filter (n = 2) was applied for noise reduction during image recording.