Aquatic Biofilms

Ecology, Water Quality and Wastewater Treatment

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Preface

On any wet surface a biofilm is easily formed, whether it is on a building, a rock in a river, marine sediments, a decaying leaf, a sewage pipe, among others. The extensive appearance of the biofilm mode of life may be linked to its properties such as nutrient entrapment and physical protection of cells from the surrounding environment. Also, this mode of life is old, underlining its resistance. It is thought that aggregated layer-structured biofilms similar to ancient stromatolites have been relevant for the origin of first microbial cells on Earth. The unique and complex characteristics of biofilms include mechanisms and processes occurring at different scales addressed by different scientific branches. Atomic forces and chemical bonds are keys for attachment processes, development of the matrix, and chemical gradients. At the cell to organisms scale, life science analyzes cell-to-cell communication, diversity of microbial metabolisms and food web interactions in biofilms. Aquatic biofilms are also a significant component at the Earth sciences scale as shown for instance by their relevance in biogeochemical cycles.

From the first report of surface-associated bacterial cells, aquatic biofilm research have been exponentially developed in the last decades, covering the study of biofilms in marine and freshwater environments, including pristine but also those affected by pollution and anthropogenic disturbances, and of those developing in man-made systems such as water engineering processes. Although in each specific environment a distinct biofilm may develop, the drivers and gradients in biofilms show parallelisms. For instance, the oxygen gradient determining specific biogeochemical reactions is similar between naturally occurring fluvial biofilms and those developing on granules for water technology purposes. Other example is the knowledge gained from anthropogenic disturbances effects on biofilms, showing parallelisms to responses observed from biofilms growing in extreme environments and developing similar resistance strategies.

The aim of this book was to compile in a single volume the latest, up-to-date theory, methodology, and applications of aquatic biofilm's research. From the theory, a broad review of biofilm history, architecture, cell communication, biodiversity and biogeochemistry is included, updating both theory and methodology. Then, the study of biofilms developing in polluted systems as well as their use and relevance as ecotoxicological sensors is reviewed. Finally, application and profit of biofilms is shown in three examples on new technologies using biofilms. We believe the different points of view and approaches presented in the book, from theory to application, from ecology to engineering, are complementary and feed from each other contributing to our understanding of biofilm mode of life.

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Glossary

The following glossary includes the definition of key concepts used among the different chapters throughout the entire book.

Alpha-diversity: the local diversity of a community contained in a habitat patch.

Aquatic biofilms: complex microbial communities attached and growing on living and non-living surfaces found in marine or freshwater or man-made environment. In biofilms microbes are attached to each other and embedded in the matrix of self-produced extra polymeric substances.

Autotroph: organism that is capable of producing nutritive organic molecules from inorganic sources, using different energy sources (light, chemical reactions).

Benthos: community of organisms living in close relationship with their substrate, in general permanently attached.

Beta-diversity: the variability of species identities among communities across space and time. Beta-diversity can occur as directional turnover along a gradient or as non-directional variation.

Bioaccumulation: general term referring to the accumulation of chemical substances, such as metals or organic pollutants, in the biota. Bioaccumulation generally provides a good proxy of the bioavailability of compounds. The term *accumulation* is often used for biofilms when the methodology does not discern between the portion of bioaccumulated chemical from the portion remaining outside of the cells, mainly adsorbed to EPS and/or inorganic particles.

Biofilm biobarriers: are physical structures where biofilm develops that are constructed to enhance bioremediation of pollutants in aqueous environments such as groundwater and surface water.

Biodiversity/Diversity: the total variety and variability among living organisms, the communities and ecosystems they are part of. Diversity measures may consider the number of species (or some other biological units), i.e. richness, their relative abundances, and the varying dissimilarities between species.

Bioelectrochemical system (BES): biological technology capable to produce electrical power or chemical compounds by the action of a biocatalyst (exoelectrogenic microorganims) generally using wastewater as anodic fuel.

Biofouling: attachment and growth of microorganisms, algae, and invertebrates on submerged surfaces. The term is specially used for man-made surfaces, such as in water distribution systems. It is also called *microbial fouling*. Such accumulation is also referred to as *epibiosis* when the host surface is another organism and the relationship is not parasitic.

Community: a group of interacting species that overlap in time and space.

Community ecotoxicology: is the study of the effects of toxicants on ecological systems focusing on the effects at community level.

Community level physiological profile (CLPP): is a rapid community-level culture approach which is used to characterize the metabolic profile of microbial communities by measuring the utilization of a range of different carbon sources.

Confocal laser scanning microscopy (CLSM): microscopy technique which allows the visualization of images at different depths of the observed sample (such as a biofilm) to compile a three-dimension final image. Confocal laser scanning microscopy may be used to detect reflection, autofluorescence signals (such as pigments of phototrophs) or the emission signals of specific fluorochromes most commonly targeting nucleic acids, lipids, carbohydrates, or proteins.

Cooperation: beneficial interactions between organisms.

Coulombic efficiency (CE): parameter used to evaluate the electrical efficiency in Bioelectrochemical Systems (BES). CE is the ratio between electrons actually transferred and the electrons potentially transferred to the electrical circuit considering the oxidation-reduction reaction occurring at the bioelectrode.

Co-diffusion and Counter-diffusion: related to the direction of diffusion of substances in biofilms. Transport of substances occurs by diffusion within the biofilm layer and is driven by a concentration gradient. In co-diffusive systems, the gradients of the electron donor and electron acceptor are parallel. On the other

hand, in counter-diffusive systems, both gradients are opposed and thus electron donor and acceptor diffuse in opposite directions.

Dispersal: the movement of individuals from one habitat patch to another. Microorganisms are assumed to have higher dispersal rates due to their high population sizes, high transportability and short generation times, which should increase their chance to reach new habitats and establish populations therein.

Dissolved organic matter (DOM): the organic fraction of the dissolved material in water (defined as those passing a 0.5 μm filter). DOM contains a mix of organic compounds (including humic substances, polysaccharides, peptides, lipids). DOM is usually quantified in carbon units and then referred as DOC (dissolved organic carbon).

Electron transfer mechanisms: the microbial mechanisms to transport and release electrons inside the membranes (intracellular electron transfer (ICET)) and to/from an electrode material (extracellular electron transfer (EET)). Different EET mechanisms are defined, such as direct (direct contact, conductive biofilm and nanowire) or indirect (mediators) extracellular electron transfer. Both mechanisms can take place simultaneously in order to maximize the microbial benefits.

Electrotrophic microorganism: group of microorganisms capable to directly receive electrons from an electrode to grow. These microorganisms are able to reduce compounds taking electrons from the electrode surface.

Epilithic biofilm: biofilm attached on rocks or cobbles. It is also named *epilithon*.

Epiphytic biofilm: biofilm growing on living plants such as that developing on macrophytes. Significant interactions with plant (which might be both synergic and antagonistic) typically occur in this biofilm type.

Epipelic biofilm: biofilm attached to the particles of cohesive sediments (clay and silt). Typical epipelic biofilms occur in intertidal areas where biofilms are mainly colonized by diatoms. It is also named *epipelon*.

Epipsammic biofilm: Biofilm attached to the particles of sandy sediments (sand and gravel). It is also called *epipsammon*.

Epixylic biofilm: Biofilm growing on dead plant material such as wood and leaves. Epixylic biofilm is mainly formed by fungi due to their ability to degrade lignocellulose compounds which are the main constituent of plant tissues. It is also called *epixylon*.

Evenness: a measure for the variation in species abundances in a community and reaches its maximum value when all species are equally abundant.

Exoelectrogenic microorganism: group of microorganisms that have the ability to transfer electrons extracellularly to the electrode material. In practical applications, exoelectrogenic microorganisms are able to oxidize organic matter from wastewater transferring electrons to the electrode material and generating electricity.

Extracellular enzymes: enzymes bound to the cell surface of microorganisms or in the periplasmatic space in gram-negative bacteria, acting outside the cell. In biofilms, free enzymes are also found within the EPS matrix. Extracellular enzymes play a key role in the decomposition of dissolved organic matter in aquatic environments since they degrade polymeric and macromolecular organic matter into low molecular weight molecules which can cross the bacterial cell membranes.

Extracellular polymeric substances (EPS): mainly composed by polysaccharides (but also by proteins, lipids, particulate material and detritus), EPS provide the mechanical stability of biofilms, mediate their adhesion to surfaces and form a cohesive three-dimensional polymer network that interconnects and transiently immobilizes biofilm cells. This matrix provides a protection from predation, toxic substances and physical perturbations.

Functional diversity: diversity of physiological or ecological units in a community. It is also a diversity measure (see Biodiversity/Diversity definition) which includes a functional notion of dissimilarity between species.

Gamma-diversity: is the diversity of a region holding multiple habitat patches with contained communities.

Granular biomass: compact granules and dense aggregates with an approximately spherical external appearance that do not coagulate under decreased hydrodynamic shear conditions and settle faster than flocs, allowing for better biomass retention and high volumetric conversion in a reactor.

Heterotroph: organism that obtains carbon from organic compounds.

Macrophytes: aquatic plants that grow in or near water. They can be floating, submersed or emerged. Aquatic macrophytes provide a substrate for algae and epiphytic biofilms.

Mass effects perspective: a meta-community perspective which emphasizes that spatial dynamics, such as source-sink effects or rescue effects, affect local community structures.

Meta-community: a set of local communities which are linked by dispersal of potentially interacting species. Several perspectives within the framework of meta-community ecology emphasize different mechanisms as potential drivers of local community composition (see the mass effect, the neutral, and the species sorting perspective definitions).

Methanogenesis: biogenic formation of methane (CH_4) as a form of anaerobic respiration in which the terminal electron acceptors are carbon compounds of low molecular weight. Methanogenesis occurring in sewers is carried out by Methanogenic Archaea (MA).

Microbially induced concrete corrosion (MICC): biological process occurring in biofilms growing on the crown of sewer pipes, surface exposed to the gas phase, that leads to the corrosion of sewers, cracking of the pipes and ultimately, structural collapse. MICC is caused by the biological production of sulfuric acid (H_2SO_4) from oxidation of hydrogen sulfide (H_2S) with oxygen (O_2) in sewers atmosphere.

Microphytobentos: synonym of the term "biofilm" but usually referred to populations of photoautotrophic microorganisms such as diatoms, euglenids, crysophyceans, dinoflagelates that colonize benthic substrata in marine systems, especially in intertidal and lower supra-tidal sediments where the light arrives.

Multichannel imaging: application of up to five separate excitation–detection combinations either simultaneously or sequentially in conventional CLSM.

Neutral perspective: a meta-community perspective, in which all species are similar in their competitive abilities. Dynamics are derived from dispersal and stochastic demographic processes.

Nestedness: degree of order/organization of a community, in which the number (due to gain and loss) of species is related to site-specific factors.

Next-generation sequencing (NGS): also known as high-throughput sequencing, is the catch-all term used to describe recent technologies (including, among others, Illumina (Solexa) sequencing and Roche 454 sequencing), which allow us to sequence relatively short DNA and RNA sequences along the entire genome much more quickly and cheaply than the previously used Sanger sequencing.

Nutrient uptake length: is the physical distance a molecule of a nutrient is transported in the flowing water until it is uptaken by microorganisms. In streams, it can be measured by adding a known content of nutrient (usually performed with phosphate, ammonia or nitrate) and follow its disappearance downstream. Nutrient uptake length is an important parameter for quantifying nutrient cycling in streams.

Nutrient stoichiometry: is the molar ratio between major nutrients (i.e. C, N and P) in organisms and in their food. The first author defining stoichiometry of aquatic organisms was Redfield who defined the molar C:N:P ratio of 106:16:1 for marine plankton.

Periphyton: synonym of the term "biofilm" and specially used in studies focusing on photosynthetic organisms.

Persistent organic pollutants (POPs): are chemical compounds that are carbon based and due to their structure possess chemical and physical characteristics that make them persistent to biodegradation in the environment for many years. They are distributed as a result of natural processes involving soil/sediment, water and air. The consequence is accumulation in fatty tissues and biomagnification in the food web, where they can cause diverse harmful effects due to their toxicity.

Phylogenetic diversity: a concept of diversity, which includes the phylogenetic dissimilarity between species, additionally to their presence/absence or abundance.

Pollusensitivity: sensitivity value of species towards levels of pollution (of various natures). This value (or the sensitivity profile of a species) is usually calculated from large datasets and describes species probability of presence in relationship with water quality.

Polychlorinated biphenyls (PCBs): are organic compounds that consist of two biphenyl rings onto which 1–10 chlorines are attached. All congeners are hydrophobic with a high biomagnification potential in the food chain, exhibit toxicity to a varying degree and a significant proportion display dioxin-like toxicity.

Quorum sensing: a form of bacterial population density depended chemical cell-to-cell communication and gene regulation.

Recovery: ability of a community under perturbation (e.g. a chemical) to restore its functional and structural attributes to initial values (measured before the perturbation) after the perturbation has ended.

Resistance: ability of a community to remain unchanged under perturbation (e.g. by the presence of a contaminant).

Sensitivity: in ecotoxicological studies it refers to the degree of modification in the function and structure of a community in response to a perturbation, such as the presence of a contaminant.

Sewers: underground network of physical structure-installations composed of pipelines, pump stations, manholes and channels that convey the wastewater from its source to the point where it is treated and discharged.

Solid retention time (SRT): is defined as the ratio between total biomass contained in a bioreactor and the rate at which biomass is washed out in the effluent. If the solid retention time is too short, slow growing microorganisms are not able to maintain their populations in the reactor and will eventually be completely washed out of the reactor.

Species sorting perspective: a meta-community perspective, which emphasizes spatial niche separation and local interactions between species and their abiotic environment.

Stable isotope probing (SIP): is a technique that is used to identify the microorganisms in environmental samples that use a particular growth substrate (i. e. organic pesticides). SIP is based on the incorporation of ^{13}C-labelled substrate into cellular biomarkers such as nucleic acids (DNA and rRNA), the separation of labelled from unlabeled nucleic acids by density gradient centrifugation, and molecular identification of active populations carrying labelled nucleic acid.

Stromatolites: ancient microbial mats which were abundant and diverse in the shallow zone of the oceans in the Proterozoic. Usually they are formed by precipitation and microbial carbonate sedimentation, resulting in a layered structure where typically cyanobacteria are present. Stromatolites played a crucial role for the early establishment of life since they consumed CO_2 and produced free O_2 and H_2.

Sulfate-reducing bacteria (SRB): group of bacteria predominant in anaerobic sewers that uses sulfate respiration to obtain energy while oxidizing organic compounds and reducing sulfate (SO_4^{-2}) to hydrogen sulfide (H_2S).

Suspended aggregates: highly fragile structures suspended in fresh and seawater made of microorganisms, organic and inorganic particles. Suspended aggregates typically occur during bloom periods after an increased input of nutrients. They are also called *lake snow*, *river snow* or *marine snow*.

Synergy: cooperation, not obligatory between microorganisms, in different processes that result in a greater benefit or production than if microorganisms were individual. In communities, cooperation helps in degradation because some strains produce the metabolites that are used for other species to complete degradation. In BESs, syntrophic interactions have been described on exoelectrogenic and non-exoelectrogenic bacteria in anode biofilms.

Syntrophic interactions: the use of one organism's metabolic intermediates and by-products by another organism as a metabolic substrate. It serves to increase internal nutrient cycling, reduce energy expenditures on resource acquisition, and can create favourable environmental conditions for novel niche development.

Taxonomic diversity: diversity based on the presence/absence or the abundance of taxonomic units, such as species, and is insensitive towards phylogenetic or functional differences among taxa.

Tolerance: the capacity of a community to support an alteration of its environment (e.g. exposure to toxicants), with no significant modifications in its structure and function.

Toxicity endpoint: is the measurement of a biological effect caused by exposure to a toxicant, usually measured as the 50% lost of the specific endpoint (i.e. metabolic activity such as organism growth, survival or reproduction, or biomass).

Xenobiotics: group of chemicals including for instance pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and antibiotics which is foreign to biological systems, but is commonly detected in the environment.

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