1 Introduction

Living in biofilms –microbial communities on interfaces– is an evolutionary successful way of life for many microorganisms. Various phenomena, like specialization, communication, etc. which are responsible for the supremacy of multicellular organisms, can also be found in microbial biofilms. Hence, biofilms are present in a multitude of ecological niches and environments: from arctic piers to hot springs, from oil pipelines to phototrophic biofilms in dolomite stone.
In the technosphere, biofilms find application in highly efficient wastewater treatment plants. The biggest challenge in this research field though is provided by unwanted biofilms - biofouling - in technical devices or even more harmful: as medical biofilms on catheters or implants where they cause persistent infections of humans. Mainly due to historical reasons biofilm research is a young discipline and detailed knowledge about biofilm development is lacking. Biofilms in their environment are highly complex systems. Numerous processes –intrinsic as well as in exchange with the environment– are taking place simultaneously. The interaction of these processes emerge in the spatial and temporal development of biofilms. In pure experimental investigations these processes cannot be separated which makes the interpretation and explanation of experimental data difficult. The central idea of this work is to integrate relevant processes of biofilm development in mathematical modeling frameworks. In the numerical solution of these models biofilm properties shall emerge virtually and are quantitatively compared with experimental results obtained under comparable conditions. Basing on this idea, an iterative method is proposed combining the use of mathematical models and their validation with data from long-term biofilm cultivations. This procedure is of an analytic nature when focusing on single processes as well as synthetic when integrating these processes in comprehensive models. Thereby, a better understanding of the processes underlying biofilm development is reached. Furthermore, this approach leads to improvements of the experimental setup and the development of two novel reactor systems in this work.
The results of comparing quantitatively simulated and experimental data are shown

and discussed. From these considerations an intensive elaboration on biofilm detachment and its mechanical causes is needed. Due to the finding of detachment phenomena to be an important but poorly understood process, a basic approach on this topic is found.

2 Preliminary comments on microbial biofilms

2.1 Biofilm basics

Biofilms are one of the oldest forms of life. The first fossil records are stromatolites which date back up to 3.5 billion years [15, 220]. They are relicts of consortia of phototrophic cyanobacteria. Precipitated or entrapped minerals in these microbial mats led to the formation of cauliflower-like rocks as displayed in **figure 2.1** [122, 123]. By producing molecular oxygen as a metabolic by-product their photosynthetic metabolism caused one of the most important changes in life. Over the millions of years, the atmosphere changed from reductive to oxidative conditions allowing the development of life as it is known today. It may become obvious in this chapter that biofilms are still of great importance and can be found in numerous environments: they are ubiquitous.

Figure 2.1: 240 million year old stromatolite as fossil record of cyanobacterial colonies (picture taken at Heeseberg, 35km south-east from Braunschweig, Germany)

Generally, biofilms can be defined as microbial communities on interfaces [160]

meaning any interface including solid-liquid, gaseous-liquid, solid-gaseous or liquid-liquid. The focus of this study are biofilms growing on solid-liquid interfaces and subjected to fluid flows. This is representative of several topics, i.e. technical (piping systems, heat exchangers), natural (benthic/riverine biofilms) and also medical (intravascular catheters) issues. Microbial communities in biofilms are enclosed in a matrix constituted by slimy extracellular polymeric substances (EPS) of microbial origin.
In numerous articles and books, biofilms are termed as for example *"...the prevailing microbial lifestyle."* [238]. It is usual to find statements like *"The fact that microbes appear to grow predominantly on surfaces..."* [115] or *"...biofilm microbiologists had concluded that bacteria grow preferentially in matrix-enclosed communities adherent to surfaces."* [38]. Even though these assertions are criticized, for example D.L. Kirchner mentions the high amount of -although low concentrated- planktonic bacterial biomass in the oceans (see correspondence in *Nature* **413**, 772; 2001), the biofilm mode of life obviously is a successful evolutionary approach of microbial life (cp. section 2.4).

2.1.1 Constituents

Surely, microbial cells are the key components of biofilms. Numerous different species of unicellular organisms from prokaryotes (bacteria, archaea) to eukaryotes (fungi, protista) are inhabiting and/or constituting biofilms. Even several metazoa (e.g. rotifers, nematodes, mites) are adapted to live in or on biofilms, respectively. However, they are by far not the only constituents. The term extracellular polymeric substances (EPS) is used to sum up all the polymers of microbial origin in a biofilm (outside the cells) [216, 25, 67]. Several different types of polymers can occur - originating from different species or even from one species under different conditions. Predominantly, polysaccharides seem to build up the biofilm matrix and constitute their structural stability but also proteins (e.g. exoenzymes: belonging to so-called active EPS), nucleic acids and lipids are found [160, 68, 129]. Extracellular DNA (eDNA) may also have a structural role in biofilm formation [241]. Furthermore, biofilms are favoring the exchange of genetic information by horizontal

gene transfer. High biomass concentrations and spatial proximity of the cells could also increase the probability for transformations (Gene transfer without conjugation by transfer of DNA through the liquid between cells). This would explain the presence of eDNA in biofilms as well. Moreover, particulate matter is found as detritus (rests of structural cell elements, discarded pili or flagella, etc.) or inorganics (e.g. trapped or precipitated minerals as of special importance for the encrustation of urethral catheters [213]). Recently, also membrane vesicles are found to be occuring in biofilms in high numbers [203]. Their function, however, is not yet definitely resolved.
The vast number of options combining the different constituents already indicates that biofilms are in the most seldom cases homogeneous and easily describable entities.

2.1.2 Biofilm structure and heterogeneity

The terms biofilm structure and heterogeneity are often used unexact. It is spoken about biofilm structure and implicitly meant heterogeneity. In this study biofilm structure shall mean the 3D spatial structure of the biofilm. For later discussions it is distinguished between three spatial scales: (1) microscale - from bacterial dimensions to the size of microcolonies (1 to several $10\mu m$), (2) mesoscale - in the dimension of biofilm thickness (up to several $100\mu m$) and (3) macroscale - in the dimension of reactor size. It is well known and also central issue of this thesis that biofilm structure is of big importance as the determinant for mass transfer at the bulk/biofilm interface [177], mechanical stability (cp. chapter 6), etc. In turn, biofilm structure is determined by environmental conditions and can also change in time [128, 29].
Following Bishop and Rittmann [16] heterogeneities in biofilms can be regarded as *"...spatial differences in any parameter we regard as important."*. They distinguish four groups of heterogeneities for which experimental evidence is found [246]: (1) *Geometrical heterogeneities* (biofilm thickness, roughness, porosity, etc.) are clearly revealed by microscopic studies (e.g. CLSM). (2) In particular, the use of microelectrodes has shown *chemical heterogeneities* for a number of compounds,

for example oxygen concentration. (3) Different reaction types are also found to be dependent on structural properties. In mixed culture biofilms Denaturing Gradient Gel Electrophoresis (DGGE) analyses reveal a high microbial diversity emphasizing *biological heterogeneity* [108]. (4) The variability of *physical heterogeneities* (density, rheological properties, stability, etc.) will be discussed explicitely in chapter 6.
The occurence of these heterogeneities is often caused by diffusion limitation of compounds into the biofilm. This leads to a stratification of the aforesaid parameters [27]. Thus, several parameters are found to depend mainly on the coordinate perpendicular to the substratum (usually z) [258, 211]. All these structural properties can change when environmental conditions (flow, substrate, pH, temperature, etc.) vary [225, 250, 134, 90].

2.1.3 Biofilm development

Development in this context generally means morphological changes of biofilms in time and/or space. Principally, it is about the temporal development of biofilms from cells initially adhering to a surface up to the formation of a mature biofilm with its specific complex structure. This can be seen as the emerging result of different processes which are partially presented in section 3.2. **Figure 2.2** shall demonstrate important stages of this development briefly[1]. Detailed treatises can be found for example in [32, 27]. Every surface in natural aquatic systems is covered by sorption processes with a (mono)layer of organic molecules, the so-called "conditioning film" (1) [32]. Effectively, this is the surface microorganisms are interacting with. Suspended microbial cells (2) can adsorb reversibly due to e.g. intermolecular forces (van-der-Waals) (3) or pili [111] (5) and also desorb again (4). After adhering irreversibly (6) the cells can proliferate, excrete EPS and thereby form microcolonies (7). From the mature biofilm (8) biomass can be released by detachment processes (9) (cp. section 3.2.3).

[1] Sincere thanks are given to Thomas Neu for providing this graphic. *http://www.ufz.de/index.php?en=1788*

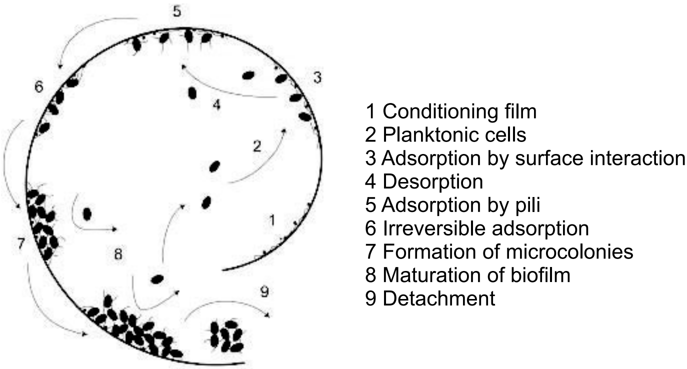

Figure 2.2: Model of biofilm development according to THOMAS R. NEU (explanations in the text)

2.2 Biofilms in nature, technosphere and medicine

2.2.1 The role of biofilms in natural ecosystems

Biofilms are present in soils, sediments, on stones, plants, animals and also humans. They can inhabit environments from glaciers to hot springs. Furthermore, they are involved in global cycles of matter (carbon, nitrogen, oxygen, etc.). Per se, biofilms have evolutionary advantages in numerous ecological niches. They can deal with varying substrate conditions and find protection against mechanical, chemical or biological attacks by their EPS matrix. The spatial proximity of different microbial species provides the possibility of forming microbial consortia. In this way complex metabolic tasks can be solved together: synergistic collaborations allow the degradation of complex substrates even under difficult conditions (e.g. absence of oxygen). Examples can be found in many places like microbial mats [74], anaerobic digestion [12] or phototrophic biofilms [247].

Solids as nutrient sources for microbes

Biofilms are predestined for the use of solids as a nutrient source. Solid organic polymers like for example cellulose must first be hydrolyzed before they can be assimilated by the cell. For this task the cell excretes hydrolytic enzymes [204]. These exoenzymes can accumulate as active EPS in the diffusion limited regime of the biofilm. Thereby the enzymes are available in high concentrations and the products of the enzymatic reaction are directly disposable for the biofilm cells [78]. Some microbes, e.g. *Geobacteriaceae* are capable of utilizing solid metal minerals as electron acceptors or energy source. Due to comparable reasons an adhered way of living is favored in order to optimize electron transport. Interestingly, pili are used as nanowires for electron transfer between cell and substratum [140, 188]

2.2.2 Biofilms in industrial applications

In process biotechnology only a few application fields are present where biofilms come to use. Artificially immobilized microorganisms or mammalian cells are not assigned to be biofilms in this sense [73]. One of the earliest processes is the production of vinegar (acetic acid) by microbial biofilms with wood chips as substratum [33]. Moreover, bioleaching in copper mining is a process in which biofilms are of great significance [24, 196, 166]. Production of bacterial polysaccharides is carried out with biofilm-forming microbes [55, 54]. The most important appearance of biofilms in industry, however, is found in detrimental aspects summed under the term "biofouling" - including numerous negative topics concerning biofilm growth in industrial plants. Fortunately, even beneficial aspects can be found as for example in wastewater treatment.

Biofouling

Biofouling is defined as the unwanted deposition and growth of biofilms [66]. Several different branches of industry like paper, chemical, or food industry suffer from biofouling. The impacts reach from a decrease in efficiency (e.g. in heat exchangers) over contamination of products (paper industry) and as far as the distribution of pathogens via drinking water distribution networks [234]. Moreover, biofilms can accelerate corrosion processes which is summarized under the term microbially influenced corrosion (MIC) [82, 174].
The strong adhesion of biofilms to a substratum makes their removal laborious and

cost-intensive. In these circumstances a consolidated knowledge might help in controlling or avoiding the growth of unwanted biofilms.

Wastewater treatment

Although trickling filters were the first wastewater treatment reactors (section 2.3), currently activated sludge systems are well established and are widely used in the treatment of municipal wastewater [245]. In biofilm reactors diffusion limitation causes a decline in reactor performance [233]. However, they provide quite a number of advantages. Not only they are stable in operation [52] and also need less installation size but also their energy demand can be much lower in comparison to that of activated sludge systems ($150 - 350Wh/m^3$ for activated sludge systems in contrast to $30 - 60Wh/m^3$ for trickling filters whereas biofilters can also reach values of more than $350Wh/m^3$) [7]. Due to the decoupling of bacterial growth rate μ and dilution rate D higher throughputs can be realized, and the flow rate is no longer creating an evolutionary pressure. This allows for the existence of slow growing organisms which are capable of degrading persistent compounds like phenols [114], naphthalenesulphonic acids [121], chelating compounds [84, 167] or others (for a detailed treatise see [83, 245]). Furthermore, the biofilm matrix can provide sorption sites which improves the biodegradability of xenobiotic compounds. Also the spatial proximity of microbial consortia favors symbiotic degradation pathways (e.g. xenobiotics, nitrification, anaerobic digestion). Higher organisms like protozoa (ciliates, amebae) or metazoa (rotifers, nematodes, mites) can better survive in the reactor by grazing on the biofilm (see also section 3.2.3) and thereby reduce sludge production. Furthermore the existence of certain rotifers (e.g. *Philodina spec.*) yields a clearer effluent.

Airlift reactors with particle fixed biomass [161, 21] have proved its value in this context because of their good mixing (good mass transfer of oxygen) and fluidisation properties. Here, inorganic particles (e.g. broken sand, [114], pumice [22], etc.) function as substratum and effect a good settlability of the biomass. But also other reactor types come to use in this field: biofilters [23, 93], rotating biological contactors [167], etc. Recently, several innovative wastewater treatment processes make use of biofilms. Enhanced biological phosphorus removal is possible with biofilm reactors [158]. Biofilms growing on gas-permeable membranes (Membrane Biofilm Reactors) can be supplied with hydrogen or oxygen gas improving for example simultaneous nitrification-denitrification [193, 219, 146]. Henceforth, a further step would be not only to get rid of undesirable compounds but also to bring them to good use for human purposes [112]. One very interesting approach is to use biofilms in the form of granular sludge in bubble column reactors. Aerobic granules in sequencing batch reactors emerge to be a promising technology for advanced wastewater treatment allowing the simultaneous removal of organics, nitrogen and phosphate [51]. Thereby feast-famine periods favor the microbial production of Polyhydroxyalkanoates (PHAs) as storage compounds [255] which are important for the formation of granules and can further be used as raw material for the synthesis of bioplastics [191].

Microbial Fuel Cells

Microbial Fuel Cells (MFCs) provide a promising technology for directly converting chemical energy to electric energy at ambient environmental conditions (concerning temperature, pressure, etc.) [186]. Recent studies reveal the important role of biofilms concerning performance and stability of MFCs [108, 139, 171]. A very interesting application is found in wastewater treatment where the biological degradation of wastewater compounds is combined with generation of electrical power [136, 81]. Nonetheless, the performance of current systems is still far from technical application. The highest area-specific power could be found in Rabaey et al. [186] with $3.6W/m^2$ substratum area which the same author lists as $216W/m^3$ reactor volume.

2.2.3 Medical biofilms

Humans and microbes live in coexistence with microorganisms, e.g. on the skin or even in symbiosis like in the gastrointestinal tract. It is well known that they can cause infections, too - needless to say also in the form of biofilms: Cystic fibrosis pneumonia is mainly caused by *Pseudomonas aeruginosa* and *Burkholderia cepacia*, biofilms of dental plaque (acidogenic gram-positive cocci like *Streptococcus spec.*)