Microbial Biofouling: Unsolved Problems, Insufficient Approaches, and Possible Solutions

Hans-Curt Flemming

Abstract Microbial biofouling is a very costly problem, keeping busy a billion dollar industry providing biocides, cleaners, and antifouling materials worldwide. Basically, five general reasons can be identified, which continuously compromise the efficacy of antifouling strategies:

1. Biofouling is detected by its effect on process performance or product quality and quantity. Early warning systems are very rare, although they could save costly countermeasures necessary for removing established fouling.
2. Usually, biofouling is diagnosed only indirectly, when other explanations fail. The common practice is to take water samples, which give no information about site and extent of biofouling deposits.
3. When finally the diagnosis "biofouling" is established, biocides are used which, in many cases, for the best kill microorganisms but do not really remove them. Killing, however, is not cleaning while frequently the presence of biomass and not its physiological activity is the problem.
4. Biofouling is a biofilm phenomenon and based on the fact that biofilms grow at the expense of nutrients; oxidizing biocides can make things even worse by breaking recalcitrant molecules down into biodegradable fragments. Nutrients have to be considered as potential biomass.
5. Efficacy control is performed again by process performance or product quality and not optimized by meaningful biofilm monitoring, verifying successful removal.

Thus, further biofouling is predictable. To overcome this vicious circle, an integrated strategy is suggested, which does not rely on one type of countermeasure,

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and which acknowledges that antifouling effects are essentially time dependent: long-term claims have to meet different (and more difficult) goals than short-term ones. An appropriate strategy includes the selection of low-adhesion, easy-to-clean surfaces, good housekeeping, early warning systems, limitation of nutrients, improvement of cleaners, strategic cleaning and monitoring of deposits. The goal is: to learn how to live with biofilms and keep their effects below the level of interference in the most efficient way.

1 Introduction

"Biofouling refers to the undesirable accumulation of a biotic deposit on a surface" (Characklis 1990). This definition is borrowed from heat exchanger technology (Epstein 1981) and applies both to the deposition of macroscopic organisms such as barnacles or mussels ("macrofouling") and to microorganisms ("microbial biofouling"). This chapter is focused on microbial biofouling.

In contrast to abiotic kinds of fouling (scaling, organic and particle fouling), biofouling is a special case because the foulant, that is the microorganisms, can grow at the expense of biodegradable substances from the water phase, turning them into metabolic products and biomass. Therefore, microorganisms are particles which can multiply. They produce extracellular polymeric substances (EPS), which keep them together and glue them to the surface and also add to the fouling. Biofouling is not only a problem in technical environments but also equally in health and medical contexts (Costerton et al. 1987). Contamination of drinking water frequently originates from biofilms and biofilm development on implants, and wounds is a common cause of serious illness and sometimes death (Gilbert et al. 2003). However, medical aspects will not be further considered in this chapter, which focuses on technical systems.

"Biofilm" is an expression for a wide variety of manifestations of microbial aggregates. Biofilms are the oldest and most successful form of live on Earth with fossils dating back 3.5 billion years and represent the first signs of life on Earth (Schopf et al. 1983). Aggregation and the association to surfaces offer substantial ecological advantages for microorganisms (Flemming 2008). Practically, all surfaces in nonsterile environments which offer sufficient amounts of water are colonized by biofilms, even at extreme pH values, high temperatures, high salt concentrations, radiation intensities and pressure (O'Toole et al. 2000; Flemming 2008). Biofilms are involved in the biogeochemical cycles of virtually all elements and are carriers of the environmental "self-purification" processes. The process is always the same: microorganisms on surfaces convert dissolved or particulate nutrients from the water phase and/or from their support into metabolites and new biomass. This is the principle of biofiltration systems used in drinking water and wastewater purification as well as many other biotechnological applications (Flemming and Wingender 2003). Of all forms of life, microbial biofilms certainly are the most ubiquitous and successful, with the highest survival potential.

Biofilms, however, can occur in the wrong place and at the wrong time. In that case, they are addressed as biofouling. It is observed in many different fields ranging from ship hulls, oil, automobile, steel, and paper production, food, beverage industries to water desalination and drinking water treatment, storage, and distribution (Flemming 2002; Henderson 2010).

In antifouling efforts, it is worthwhile to keep in mind that biofilm organisms have developed effective, versatile, and multiple defence strategies over billions of years against a multitude of stresses, including those caused, for example, by heavy metals, irradiation, biocides, antibiotics, and host immune systems. Therefore, an easy and lasting victory over biofouling cannot be expected.

Furthermore, it has to be considered that antifouling success is time dependent and not permanent. Sooner or later, all surfaces will be colonized by microbial biofilms. Kevin Marshall, one of the key researchers of early biofilm research once commented antifouling efforts: "The organism always wins" and he is right. The question is only how long the time span of nonfouling can be extended. The temporal requirements vary from hours to days (e.g., removable catheters, food and beverage industry, pharmaceutical industry) to months and years (e.g., desalination membranes, ship hulls, and environmental sensors). This makes it difficult to extrapolate short-term experiment results to long-term success.

2   The Costs of Biofouling

Biofouling is a costly problem. Although it is a common phenomenon, there is little quantitative data about the caused costs. Admittedly, it is very difficult to assess such costs as they are composed by a number of various factors: from interference with process performance, decrease of product quality and quantity, to material damage by microbial attack which even can include minerals (Sand and Gehrke 2006) or metals (Little and Lee 2007), preventive overdosing of biocides and cleaners, and finally, most expensive, interruptions of production processes and shortened life-time of plant components due to extended cleaning. An additional matter of expense is represented by treatment of wastewater contaminated by antifouling chemicals.

Collectively, biofouling causes considerable damage and supports an economically healthy antifouling industry offering everything from antifouling surfaces and materials, biocides, cleaners, and consulting services – this market is worth billions of dollars annually worldwide, considering the volume of the biocide divisions of major chemical companies. There are two reasons for this (1) the dimension of the problem with so many industrial areas concerned, and (2) the poor efficacy of many antifouling efforts which requires frequent and ongoing countermeasures.

As an example for a cost assessment, Flemming et al. (1994) estimated the costs of biofouling in a membrane application at Water Factory 21, Orange County, to 30% of the operating costs, at that time about $750.000 per year – such a rate has not much changed since. The estimate considered not only on the costs for membrane

cleaning itself and labor costs but also down-time during cleaning, pretreatment costs, including biocides and other additives, an increased energy demand due to higher transmembrane and tangential hydrodynamic resistance, and shortened lifetime of the membranes. In a case study, the author is currently involved in treatment of seawater for injection into oilfields for replacement of the oil was performed by nanofiltration membranes. The client reports: "Due to biofouling, membrane life is reduced from 3 to 1 year, so over the life of the plant the cost of membrane replacement will be increased by 3. If it is taken into account that each membrane costs 2500 € and each plant has around 700 membranes, one can easily calculate a yearly investment cost of 1.75 million instead of 0.58 million. That means an extra cost of 1.17 million a year just for membrane replacement, but this can easily increase significantly if man hours involved in replacements, filters and piping replacement cost, fees paid to the client for downtimes and low quality water and further factors are taken into account." Such cost assessments, even if crude, reflect how complex and essentially arbitrary any numbers are, but they show one thing for certain: that they are high. In particular, downtime caused by biofouling amounts to surprisingly high overall costs: Azis et al. (2001) estimated the costs for biofouling in desalination to 15 billion US$ yearly worldwide.

In heat exchangers, the decrease of efficacy of heat transfer is the first aspect of biofouling-related costs and contributes to the "fouling factor" (Characklis et al. 1990; Zhao et al. 2002; Hillman and Anson 1985; Flemming and Cloete 2010). Biofouling – very conservatively assumed – accounts for about 20% of overall fouling in energy generation. To match the fouling factor, preventive extended dimensioning of heat exchanger plants is a common practice. Thus, biofouling directly increases the capital costs of, for example, a power plant (Murthy and Venkatesan 2009). In power plants around the world, thousands of tons of chlorine are spent each day to combat biofilms, which amounts to high values in terms of biocide and wastewater treatment costs (Cloete 2003). Again, down-time for cleaning causing loss of production and labor costs contribute a much larger share of costs.

Treatment of wastewater contaminated with antifouling additives represents an emerging cost factor as the release of biocides is increasingly restricted and will cause more effort for removal – a problem which will come further into focus in Europe when new EU guidelines which limit the biocide content in effluents come into action (Flemming and Greenhalgh 2009; Cheyne 2010). What clearly makes more sense is putting more effort in prevention of biofouling by advanced strategies.

In marine environments, the primary cost factor of biofouling is the increase in drag resistance, for example, on ship hulls (Schultz 2007; Edyvean 2010) or on heat exchanger surfaces (Andrewartha et al. 2010). Marine biofouling begins with the adhesion of microorganisms which form a microbial biofilm ("slime") to which other organisms may adhere, settle, and grow (Stancak 2004). Characklis (1990) calculated that on ship hulls, a biofilm with a thickness as small as 25 µm can increase drag by 8% and a roughness element of 50 µm will increase drag by as much as 22%.

3 Biofouling: An Operationally Defined Parameter

As indicated earlier, the natural phenomenon underlying biofouling is biofilms. The term "Biofouling" is operationally defined and is not determined by any objective scientific reason and standard, but only on process efficiency considerations. All nonsterile technical water systems bear biofilms, but not all of them suffer from biofouling. Therefore, a threshold level must exist above, which biofouling begins. This "level of interference" (a.k.a. as "pain threshold") is defined mostly by economical considerations defined by the extent to which biofilm effects can be tolerated without inacceptable losses in process performance or product quality and quantity (Flemming 2002). Beyond this point, which can be quite different in various industries, biofouling begins. This can be illustrated by the well-known logistic curve as shown in Fig. 1.

This threshold of interference is a felt limit, which reflects the fouling tolerance of an operator. Although it may be felt differently in different technical fields, it is safe to assume that eventually a 30% loss of productivity, product quality loss, or process efficacy will alert any operator who will try to identify and eliminate the reason. Then, usually a vicious circle begins which is sketched in Fig. 2. The main stations in that circle are (1) indirect detection of biofouling by product or process quality loss, (2) indirect and not very robust verification of biofouling, (3) no nutrient limitation although nutrients are potential biomass, (4) more or less blind use of biocides instead of cleaning, and (5) no proper verification of remedial action.

Virtually, every industrial field which has to struggle with biofouling has developed its own "culture" to handle this problem, and there is not much lateral
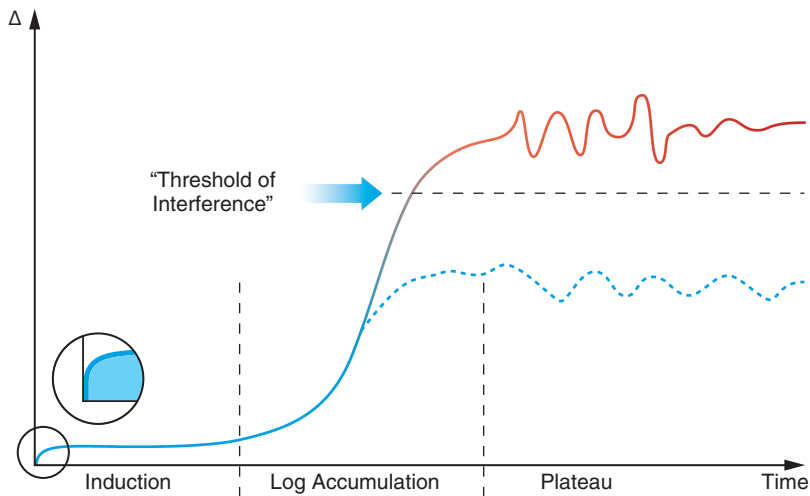

Fig. 1 Development of biofilms below and above the "threshold of interference". Δ = Parameter for biofilm effect, for example, friction resistance, hydraulic resistance, thickness, etc. (after Flemming and Ridgway 2009) *Inset*: Primary adhesion
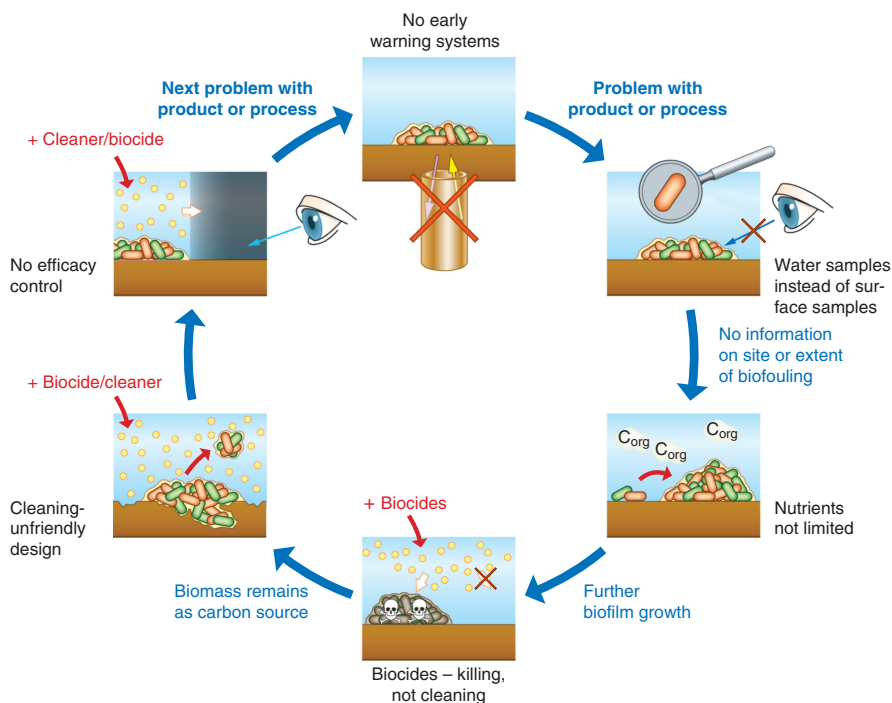
Fig. 2 The vicious circle of conventional anti-fouling efforts

learning from other fields. For example, antifouling systematics in food, beverage, pharmaceutical and microelectronics industries (Cole 1998; Verran and Jones 2000; Wirtanen and Salo 2003) are much more advanced compared to the state of the art in other biofouling-concerned technologies such as power generation (Henderson 2010), membrane treatment (Flemming and Ridgway 2009), or process water use in automobile, paint or cosmetics and medical products manufacture.

4 An Integrated Antifouling Strategy

Breaking this vicious circle is not possible with one-shot solutions but rather by proper process analysis and integrated, holistic approaches – which still represent rare exercises in the field. This chapter is intended to contribute to such an approach.

4.1 Detection, Sampling, and Analysis of Biofouling

As already pointed out, usually, biofouling is diagnosed only if problems in product quality or process performance occur, which cannot be explained by conventional

technical or chemical reasons. Then, it is a common practice to take water samples at points of use of the water and determine the number of planktonic bacteria. The result may be quite misleading as numbers of planktonic bacteria neither indicate location nor extent of biofilms in a system. This leads to the generation of piles of useless data, which is frequently observed in practice. Early warning systems (see Sect. 4.5) are usually missing.

However, if taken systematically, water samples still can indicate hot spots of biofouling in a technical system. An example from practice was a water purification system in which microbial counts were determined from water samples after intake reservoir, flocculation, and filtration units were low but after the ion exchanger unit they increased for three orders of magnitude. This revealed the ion exchanger as origin of the biofouling problem. Thus, systematically upstream water sampling can lead to foci of biofouling.

Then, it is a good idea to take surface samples, preferably from defined surface areas, and to analyze them in the laboratory (Schaule et al. 2000). Quantification of microorganisms is usually performed by determination of viable counts ("colony-forming units," cfu). However, they reveal only the "tip of the iceberg" because the proportion of cultivable organisms in environmental and technical microbial populations is usually less than 1% of the actual total number of bacteria present in the sample (Rompré et al. 2002). Most cells, particularly in the depth of biofilms, do not multiply on the commonly employed nutrient agars and, thus, will not be detected by cultivation methods. Nevertheless, they are part of total biomass and in cases where the physical properties of biomass cause the problem, it makes perfect sense to determine the total microbial cell numbers by fluorescence microscopy (Schaule et al. 2000). If the proportion of cultivable bacteria (cfu) is high, for example, 10–100%, it indicates the presence of nutrients and a high biofouling potential (Wingender and Flemming 2004).

Another point to be considered is the frequent coincidence of biofouling with nonbiological fouling. Figure 3 shows an example of mixed fouling on the feed side of a reverse osmosis membrane, eventually blocking it beyond cleaning. In hindsight, it is always difficult to tell what came first. But it is well known that biofilms promote the precipitation of minerals (Arp et al. 2001; van Gulck et al. 2003). EPS components such as polysaccharides seem to play a crucial role in such processes (Braissant et al. 2003; Flemming and Wingender 2010).

4.2 Low-Fouling Surfaces

One of the most obvious targets in antifouling strategies is the selection or development of surfaces, which are not readily colonized by microorganisms and, ideally, easy to clean. Such surfaces are referred to as "low fouling." Clearly, rough surfaces are more prone to microbial colonization than smooth surfaces. This has been confirmed with stainless steel surfaces: even on the smoothest surface, bacteria can attach.
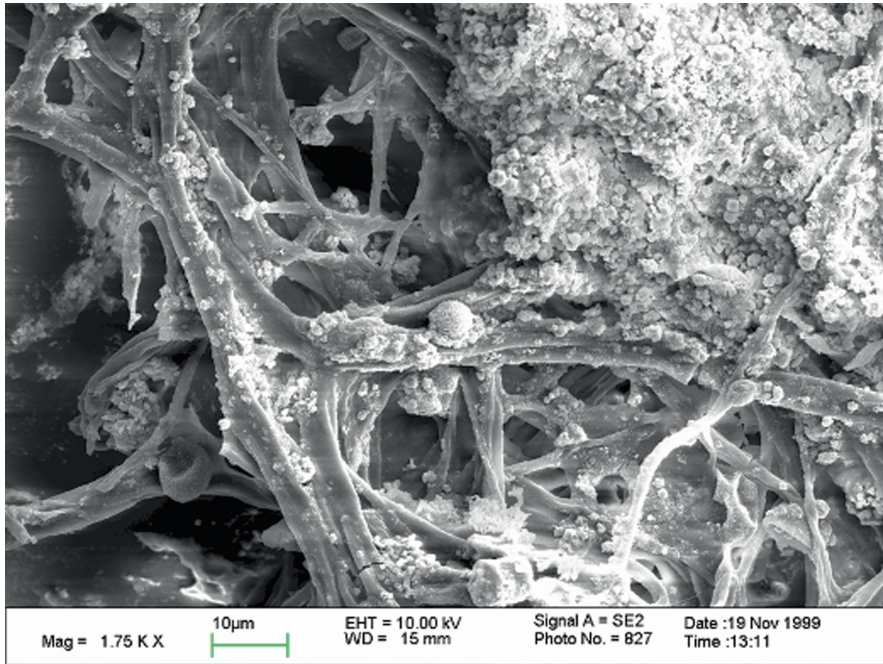
Fig. 3 Mixed microbial and abiotic deposit on a terminally fouled reverse osmosis membrane feed water surface (courtesy of G. Schaule, IWW Mülheim)

This is the result of unsuccessful approaches to prevent biofouling in heat exchangers by electropolishing (Characklis 1990; Jullien et al. 2003).

It is worth to take a closer look into the scenario of a microorganism approaching a surface prior to adhesion, which is schematically depicted in Fig. 4. A surface submerged in water will first be covered by a conditioning film, a long known phenomenon (Baier 1982). This is the result of the "race to the surface" of all molecules and particles present in the water phase, even at very low concentrations. Biopolymers meet surfaces prior to bacteria. This is due to the fact that bacteria simply do not move as fast as molecules in the water phase. Once in contact, they tend to be kept to the surface by a multiple hook-and-loop mechanism, provided by weak physicochemical interactions such as hydrogen bonds, van der Waals and weak electrostatic interactions at contact with the surface. Biopolymers have a very high number of possible binding sites, for example, polarized bonds, OH groups, or charged groups. If only 1% of them interact with a surface, the overall binding energy can exceed that of single covalent bonds by far. The molecules may migrate and spread out on the surface and attach irreversibly. Eventually, the conditioning can partially mask original surface properties. Cells in suspension usually are surrounded by a more or less thick layer of EPS and, for some organisms, cellular appendages such as fimbriae and pili. These molecules and appendages are sticky and make first contact to surfaces, interacting with both conditioning film and the
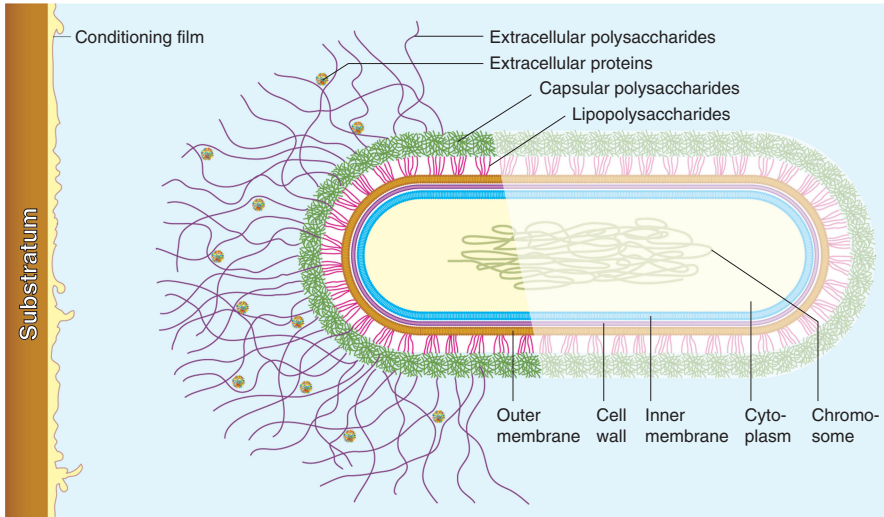
Fig. 4 Schematical depiction of a Gram-negative bacterium approaching a submerged surface

surface itself. The cells do not need to be viable for adhesion as the phenomena is controlled by physicochemical interactions.

Already in 1971, Marshall et al. tried to understand microbial primary adhesion as the interaction between "living colloids" and surfaces, applying the theory of Derjaguin, Landau, Vervey, and Overbeck (DLVO), a concept which was further evaluated for a long time (Hermansson 2000), but it clearly did not allow for realistic predictions as recent research confirmed (Schaule et al. 2008). Obviously, this approach does not acknowledge all factors involved in primary adhesion, in particularly not the role of EPS, or cellular appendices.

Many approaches have been pursued to prevent biofilm formation, some of which may be critically discussed here:

1. *Tributyl tin antifouling compounds*. They are extremely successful in biofouling prevention and have been widely used in antifouling paints for ships (Howell and Behrends 2010; ten Hallers-Tjabbes and Walmsley 2010). However, they are so toxic to marine organisms that they have been widely banned from use, although they were considered as "wonder weapons" for quite some time. It perfectly fulfilled its purpose from an antifouling point of view but it caused inacceptable economical and environmental damage (Maguire 2000; van der Oost et al. 2003).

2. *Natural antifouling compounds*. Such compounds have been isolated mainly from marine plants, which are practically not colonized by bacteria (Terlezzi et al. 2000). De Nys et al. (2006, 2010) have isolated signalling molecules from an Australian seaweed, exhibiting activity against bacterial colonization. More marine antifouling products have been investigated by Armstrong et al. (2000). Turley et al. (2005) used pyrithiones as antifoulants. Dobretsov (2009) concentrated on quorum sensing molecules as targets to prevent biofouling,

suggesting using quorum sensing inhibitors for biofouling control. In their review, they present an overview on the wide variety of quorum sensing molecules. Unfortunately, not all biofilm organisms can be addressed by one single quorum sensing inhibitor. The problem with natural antifouling compounds is that (1) most of them are only scarcely available, (2) that they are difficult to apply on a constant basis on a surface, (3) they do not completely prevent biofilm formation on inanimate surfaces, (4) that they will select for organisms which can overcome the effect, and (5) they are inherently biodegradable and, thus, their effect can be short-lasting. Apart from that, they will have to undergo the EU biocide guideline procedure, which is assessed to cost about 5–10 million € per substance (see Flemming and Greenalgh 2009; Cheyne 2010).

3. S*urfaces with lotus effect* (Nienhuis and Barthlott 1997; Marmur 2004). This effect relates to the "purity of the sacred lotus" (which is shared by less sacred cabbage leaves as well) based on the particular structure of the wax layers on the leaf surface. A highly hydrophobic pattern of needles in micrometer distances will prevent water from moistening the surface due to the physicochemical interactions of three phases: solid, liquid, and gaseous. By nature, this effect is not possible with immersed surfaces. Also, as soon as surface active substances cover the hydrophobic pattern, surface tension decreases and water is no longer repelled. Thus, the lotus effect can be taken advantage of only on solid–air interfaces and only if no surfactants are used.

4. *Silver-coated surfaces*. These are presently very much favoured with many reports on decreased adhesion (e.g., Gu et al. 2001; Gray et al. 2003; de Prijck et al. 2007). The efficacy of silver deserves critical considerations. It is still unclear whether this is an antideposition or antimicrobial effect. With regard to the question of microbial response, the Ag^+-ion is generally accepted as the effective agent (Silver 2003). The most important problem with silver efficacy is that the organisms will develop resistance after extended exposure. In environmental systems, occurrence of resistance has to be expected rather sooner than later, that is in terms of some weeks or a few months (Flemming 1982; Silver 2003). Furthermore, reactions of the silver ion with abiotic compounds have to be considered in open systems, which decrease their active concentration. Thus, silver resistance seems to be widely underestimated while its efficacy is even more overestimated.

5. *Surface-bound biocides*. Biocides attached to surfaces, such as already suggested by Hüttinger et al. (1982) and continue to be investigated and patented in many versions (see Table 1), all are suspected to draw their efficacy from biocides leaching into the water phase. If this is excluded, some basic questions have to be considered:

   – What happens with bacteria which are killed by contact and essentially will cover the surface?
   – How such biocides may act, because by concept, they do not enter the cytoplasm;

Table 1 Examples for approaches to minimize primary biofilm formation by surface modifications

Principle	References
Smoothing of surfaces	Whitehead and Verran (2009)
Superhydrophilic surfaces	Vladkova (2009)
Superhydrophobic surfaces	Schackenraad et al. (1992), Marmur (2004), Genzer and Efimenko (2006), Whitehead and Verran (2009)
Microstructured surfaces	Bers and Wahl (2004), Carman et al. (2006)
Si- and N-doped carbon coatings	Zhao et al. (2002)
UV-activated TiO_2 coatings, Ag nanoparticles	Sunada et al. (2003)
Pulsed surface polarization, pulsed electrical fields	Perez-Roa et al. (2006), Schaule et al. (2008), Giladi et al. (2008)
Polyether-polyamide copolymer (PEBAX) coating	Louie et al. (2006)
Low-surface energy coatings	Vladkova (2009)
Surface conditioning	Marshall and Blainey (1990)
Incorporation of antimicrobials	Whitehead and Verran (2009)
Biocides directly generated on surfaces	Wood et al. (1996, 1998)
Surface-bound biocides and antimicrobial peptides	Hüttinger et al. (1982), Tiller et al. (2002), Milovic et al. (2005), Madkour et al. (2008), Zasloff (2002), Leeming et al. (2002), Parvici et al. (2007), Lewis and Klibanov (2005), Park et al. (2006), Klibanov (2007)
Combined, multiple approaches	Majumdar et al. (2008)

- – Do they also act on cells which attach but are physiologically inactive (e.g., in the viable-but-noncultivable state? Oliver 2005, 2010)
- – How can deposition of abiotic foulants on these surfaces be prevented? (Webster and Chisholm 2010)

6. *UV irradiation*. The use of UV irradiation is also discussed for fouling control (e.g., Patil et al. 2007). However, it has to be taken into consideration that UV irradiation can only kill those cells, which are exposed to the UV rays. They can be shielded by particles while passing the irradiation chamber. Furthermore, the efficacy of UV light against biofilms is more than doubtful, in particular, if the biofilm has trapped particles. And even if the biofilm bacteria are killed, they provide nutrients for others and they are not removed from any surface.

There is a vast variety of further approaches to prevent adhesion or at least to minimize it and slow down biofilm formation. Table 1 is listing only a few of them – it is essentially incomplete because this field is one of the most innovative and under continuous development, generating hundreds of publications every year. Among those, the review of Meseguer Yebra et al. (2004) is particularly interesting as it is dedicated to environmentally friendly antifouling coatings. Many innovations come from the field of marine technology (Finnie and Williams 2010). Concerns over the environmental impact of antifouling biocides (Howell

and Behrends 2010; ten Hallers-Tjabbes and Walmsley 2010) have led to interest in the development of biocide-free control solutions (Finnie and Williams 2010).

There are some general problems, which have prevented the expected breakthrough of such approaches so far, in spite of the sometimes enthusiastic and advertising character of some of the publications. There are some sobering general problems:

1. The duration of the effect. In many cases, the tests are carried out only for a few hours or days. If such approaches are applied to surfaces which are to be protected only for a short time, it may be sufficient, but mostly not for long time applications. It has to be taken into account that fouling protection is a matter of time – extrapolation from short periods to longer ones is usually not valid.
2. The test system itself. In many cases, *E. coli, P. aeruginosa* or other standard organisms are used, mostly as single strains and after washing, which leads to very unrealistic conditions with the actual sticky components at least partially removed by the washing process. Then, the number of colony-forming units (cfu) per surface area usually is the parameter to determine success, although it might be taken into account that the cells may react to contact to some of these surfaces, becoming noncultivable (Flemming 2010). Therefore, cfu numbers will be lower than the numbers of actually present cells and success is overestimated.
3. A further problem with all antimicrobial coatings in technical or environmental systems will be the covering by abiotic compounds such as humic substances, oil etc., and by inactivated cells sooner or later, masking the original effect. An example: copper-plating of ship hulls (Howell and Behrends 2010) only extents the phase until copper-tolerant microorganisms completely cover the surface and allow less copper-tolerant organisms to settle on top, eventually leading to biofouling. A longer lag phase of biofouling, however, may be very valuable, as long as the time limitation of this phase is taken into account, because time is a crucial factor in efficacy of such surfaces.

An interesting novel approach may be the employment of environmentally responsive polymers (Ista et al. 1999). An example is the use of pulsed polarized surfaces. This is also in its early experimental development but may open an interesting window in mitigation of biofouling. Schaule et al. (2008) used surfaces coated with indium-tinoxide (ITO) and polarized them at ± 600 mV under potentiostatic conditions in a pulsing routine of 1 min. Originally, this approach was intended to prevent microbial adhesion but failed to do so. However, unexpectedly, it significantly inhibited biofilm development after primary adhesion of microorganisms (Fig. 5). Of course, all arguments as mentioned above are valid for these approaches equally.

4.3 Limitation of Biofilm Growth

Given the fact that it is very difficult to prevent biofilm formation on a long term, the next plausible approach is to limit the extent of biofilm growth to limit its growth-related effects. The most obvious approach is to keep the nutrient content as low as possible, both in the water phase or leaching from the substratum. Carbon
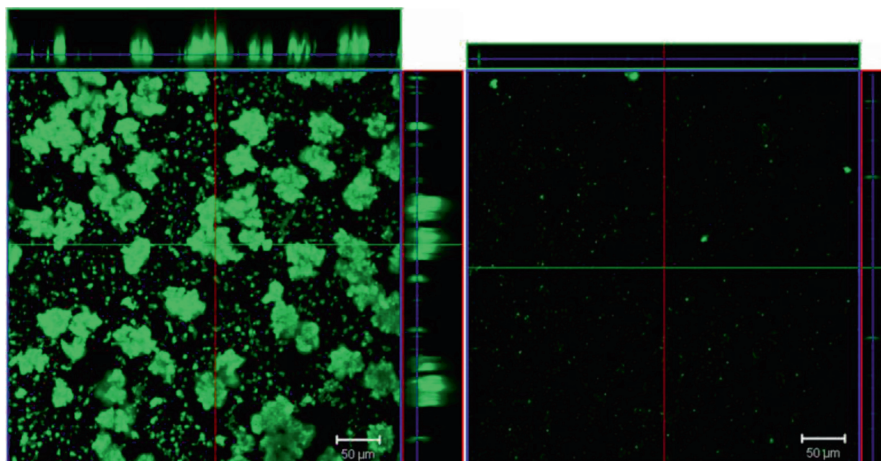
Fig. 5 Biofilm development after 164 h under potentiostatic conditions (−/+600 mV, pulse frequency 60 s), control (left), polarized biofilm (right) (Schaule et al. 2008)

Table 2 Effect of sand filtration on biofilm development on a flat cell membrane (Griebe and Flemming 1998)

Parameter	Unit	Before filter	After filter
Total cell count	[cells/cm^2]	1.0×10^8	5.5×10^6
Colony count	[cfu/cm^2]	1.0×10^7	1.2×10^6
Protein	[µg/cm^2]	78	4
Carbohydrates	[µg/cm^2]	26	3
Uronic acids	[µg/cm^2]	11	2
Humic substances	[µg/cm^2]	41	12
Biofilm thickness	[µm]	27	3
Flux decline	[%]	35	<2

sources have to be considered primarily, although lifting of nitrogen and phosphorus limitation may also be a reason for increased biofilm formation. If biofouling can be considered as a "biofilm reactor in the wrong place" as pointed out earlier, it is logical to use a "biofilm reactor in the right place." The "right place" is ahead of any system to be protected. For the case of nutrients in water, this has been successfully implemented to prevent biofouling in membrane systems (Griebe and Flemming 1998, Table 2) and is increasingly applied now in membrane technology and also in the protection of heat exchangers against biofouling. It does not completely eliminate biofilm growth but allows for keeping it below the threshold of interference as depicted in Fig. 1.

Figure 6 shows thin cuts of the membrane and biofilms (a) before and (b) after the sand filter in the above cited study. Clearly, the protected membrane was not free of biofilm but the effect of this much thinner biofilm was below the threshold of interference. It is not necessary to kill or remove such biofilms but well possible to live with them.
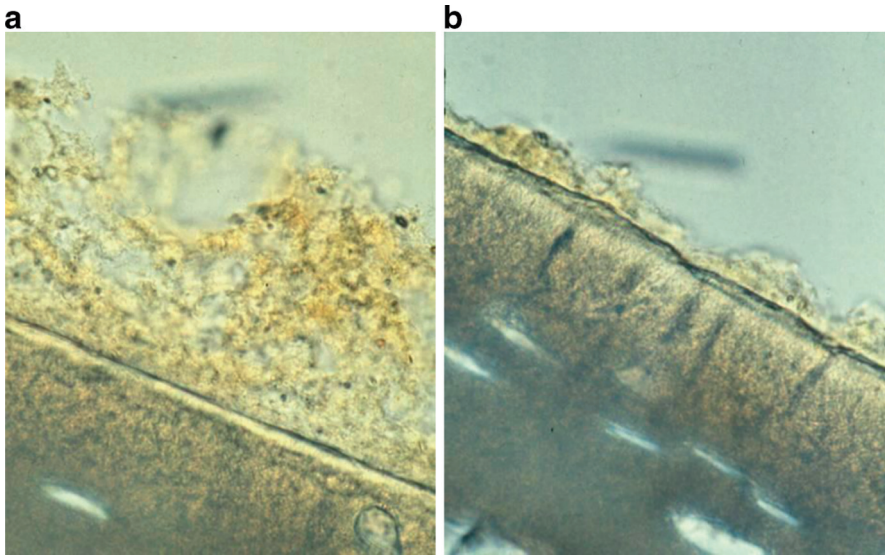
Fig. 6 (**a**) Biofilm on a reverse osmosis membrane before sand filter, (**b**) after sand filter. Magnification: 400 fold. (Griebe and Flemming, unpublished)

Of course, in this context it has to be taken into account that some additives (e.g., antiscalants, flocculants, phosphate, biodegradable biocides and components of synthetic polymeric materials such as plasticizers, anti-oxidants and flame retardants) can unintentionally contribute to nutrient supply and support biofilm growth. This has been observed in biofouling case histories of water distribution systems (Kilb et al. 2003).

Maintenance of high shear forces also helps to limit the extent of biofilm growth; however, it may lead to thinner but mechanically more stable biofilms (Characklis 1990) because higher shear forces will select for EPS with higher cohesion forces and wash away those polymers which cannot stick to the matrix.

Limitation of the extent of biofilm development seems to be an equally obvious as neglected aspect in antifouling strategies. However, it may be one of the most pragmatic approaches and can be applied creatively, adapted to the system to be protected, if taken into account. Of course, this is not generally applicable but if it is applied where it is possible, it leads to considerable success. It simply requires a shift of thinking from the "medical paradigm" toward the use of understanding of biofilm dynamics (Flemming 2002).

4.4   Biocides Versus Cleaning

A reason for frequent antifouling failures is the "medical paradigm" on which current common antifouling measures are based: biofouling is considered as a

kind of a "technical disease," caused by microorganisms and can be "healed" by killing these, using biocides. This is usually called "disinfection," although it does not fit into the proper definition of this word. However, biofouling is commonly not the result of a sudden invasion of microorganisms but of a more or less rapid deposition and growth. In many cases, that is due to an increase of accessible nutrients and can occur quite unexpectedly, even after application of oxidizing biocides. A consequence of the medical paradigm is the expectation that killing of the organisms will solve the problem. However, in the first place, it is surprisingly difficult to kill biofilm organisms (Schulte et al. 2005). They have developed many ways to tolerate biocide concentrations, which would kill suspended organisms easily (Gilbert et al. 2003). The common way to determine the success of biocide application by cultivation methods will not reveal if biofilm has been removed or if it still remains as dead biomass on biofouled surfaces. This can only be determined by parameters, which actually reflect biomass, for example by direct enumeration of cells using fluorescence staining of nucleic acids. The difference is illustrated in biocide application experiments inspired by practice in heat exchanger. In an annular rotating reactor as described by Lawrence et al. (2000), a biofilm was grown from a drinking water population and run with drinking water enriched by 0.1% CASO broth as nutrient source. It was repeatedly treated with a combination of hydrogen peroxide and peracetic acid (28 ppm H_2O_2, 1.2 ppm peracetic acid for 1 h). The results are shown in Fig. 7 (Schulte 2003).

The colony counts indicate a significant reduction in "living" bacteria, which usually would have been interpreted as a substantial biomass removal. But microscopic enumeration of total cell numbers revealed that most of the biomass still remained on the surface. The cells simply did not multiply in the cultivation assay
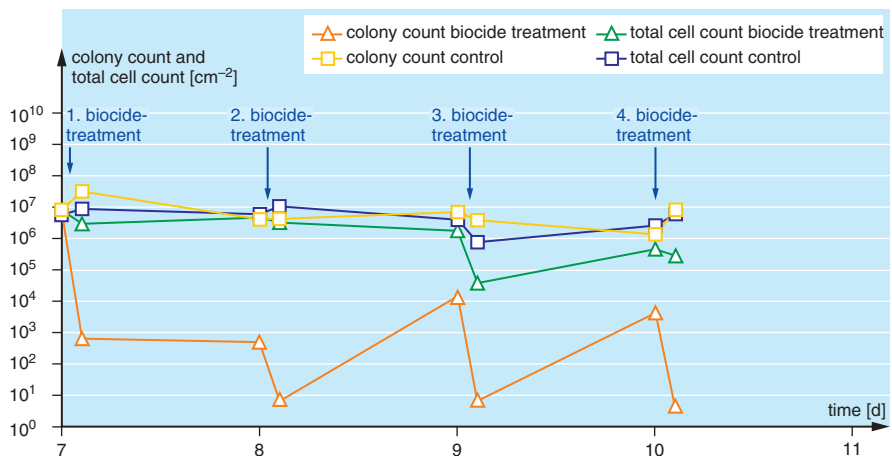

Fig. 7 Treatment of biofilm grown in an annular rotating reactor with combined hydrogen peroxide and peracetic acid (28 ppm/1.2 ppm) for 1 h. Quantification of microorganisms by cultivation (cfu) and total cell determination by fluorescence staining with DAPI (Schulte 2003)

used for their quantification. And obviously, they quickly recovered after every biocide treatment step.

The quantities of biocides and/or cleaners applied to control biofouling are mostly based on "gut feeling" instead of indicative data and specifically targeted measures. It is a disconcerting fact that the state of art in cleaning still is more an art than a science. Although serious research is performed in terms of surface cleaning, the application of this research has not yet gravitated to practice. Arbitrary mixtures of complexing substances, enzymes, shock dosages of oxidizing and nonoxidizing biocides, pH-shocks and others are common practice.

Killing is not cleaning – this has to be taken into account, because in technical processes such as cooling systems or water filtration, biomass itself, regardless if alive or dead, still causes the problems. It does not help to only inactivate a part of the population – which will soon recover after the biocide is rinsed out. The most advanced cleaning concepts have been developed in the food industry (Wirtanen and Salo 2003; Whitehead and Verran 2009). Here, surfaces continuously become contaminated by food components, which have to be removed. Much of the work is dedicated to protein adsorption and desorption (Vladkova 2009).

4.4.1   Mechanical Cleaning

Mechanical cleaning of biofouled surfaces is probably the oldest and most successful. Brushing teeth may serve as a metaphor, which also illuminates the fact that it is not possible to mechanically clean surfaces once and forever. But the analogy also illustrates that timely, properly carried out cleaning is quite efficient. In technical environments, it is used in many different applications, including "pigs" as mechanical plugs of various configurations to remove deposits in pipelines. In the food industry, ultrasonic treatment has been reported for some applications as a successful means of keeping surfaces clean (Boulangé-Petermann 1996). Wu et al. (2008) suggested defouling by use of nanobubbles. A macro version of this cleaning method is based on the use of air–water flushing which is for example used for the cleaning of drinking water pipes or in membranes (Cornelissen et al. 2007) or the combined use of air scouring and sponge ball cleaning (Psoch and Schwier 2006). Of course, there remain open questions about the accessibility of surfaces and the energy demand, but for specific purposes this method may be suitable.

An aspect crucial for cleaning success is system design. Pipes can vary in diameter, have many bends, branches and even dead legs, and can consist of a wide variety of materials. For some sections, pigging may be possible but only if the system is suited for by design – for the rest, chemical cleaning will be the only choice (Cloete 2003). Part of the problem with cleaning is the fact that biofouling is not always homogeneously distributed in a system and can be focused at certain sites, for example, fittings, valves and especially air–water–solid interfaces. Therefore, information about the actual location of biofouling foci is required but usually

missing and mostly not even aimed for. To pinpoint the location, a systematic sampling approach is required. Access to surfaces is a great advantage and should be considered in construction from the very beginning. Usually, this implicitly leads also to a less fouling-prone system, avoiding dead legs, tortuous piping with various diameters and rough surfaces.

However, in spite of the efficacy of mechanical cleaning methods, it selects for biofilms with strong cohesive and adhesive properties. This has been shown in early work on ocean thermal energy recovery (OTEC) when repeated mechanical cleaning led to very sticky biofilms which (Nickels et al. 1981), which were resistant to any chemical cleaning and could only be removed mechanically.

4.4.2   Chemical and Biochemical Cleaning

The main requirement of a cleaner is to overcome the adhesion of the biofilm to the surface and the cohesion forces, which keep the biofilm together to disperse it. The mechanical stability of biofilms is mainly attributed to weak physicochemical forces such as hydrogen bonding, weak ionic interactions, hydrophobic and van der Waals interactions and entanglement (Mayer et al. 1999; Flemming and Wingender 2010 a). While surface active substances mainly address hydrophobic and van der Waals interactions, complexing substances act on ionic bonds. Hydrogen bonds can be addressed by so-called chaotropic agents such as urea, tetramethyl urea and others which interfere with the shell of water molecules surrounding biopolymers.

Matrix stability provided by entanglement of the biopolymers (Wloka et al. 2006) can be weakened by either oxidizing biocides or by enzymes, both shortening the chain length of the polymers. Enzyme applications in the food industry have been extensively studied (Lequette et al. 2010). In another very recent publication, Kolodkin-Gal et al.(2010) reported biofilm disassembly in *B. subtilis*, *P. aeruginosa*, and *S. aureus* by a mixture of D-amino acids, releasing amyloid fibers that linked the cells together. Bacteriophages induce a wide range of polysaccharide-degrading enzymes in their hosts. Dispersion by induction of a prophage, followed by cell death and subsequent cell cluster disaggregation has been observed (Webb et al. 2003). However, phage enzymes are very specific and rarely act on more than a few closely related polysaccharide structures. Phages and bacteria can coexist symbiotically within biofilms, suggesting that they would make poor tools for the control of biofilm formation. Combinations of phage enzymes and disinfectants have been recommended as possible control strategies under certain conditions (Tait et al. 2002) with the phage added before addition of disinfectant being more effective than either of these alone. Mixtures of enzymes are commonly used, composed on arbitrary base. However, Brisou (1995) already showed that there were a vast variety of target structures that enzymes had to interact with, indicating that there is no single enzyme or enzyme mixture to effectively remove biofilms. Klahre et al. (1998) report poor performance of enzymes alone in antifouling efforts in paper mills, particularly in long-term applications. The enzymes themselves are rapidly degraded by extracellular proteases.

An interesting, but possibly overestimated approach to get rid of biofilms is the employment of signalling molecules regulating biofilm development are prime targets for biological biofilm removal (Webb et al. 2003). In technical systems, however, there are no reports of successful applications so far. Recently, a substituted fatty acid, *cis*-11-methyl-2-docecenoic acid, called "diffusible signal factor" (DSF), was recovered from *Xanthomonas campestris*, which was responsible for virulence as well as for the induction of release of endo-β-1,4-mannanase which degrades mannose containing polysaccharides (Dow et al. 2003). Davies and Marques (2009) suggested the activation of stress regulons, which may be involved in biofilm dispersion. They reported *cis*-2-decenoic acid as a fatty acid messenger, produced by *P. aeruginosa*, capable of inducing dispersion of biofilms formed by *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *S. pyrogenes*, *S. aureus*, *B. subtilis*, and the yeast *C. albicans*. Such a "universal biofilm disperser" is of great interest in medical and technical systems. Equally recently, the role of nitric oxide has been revealed as signal for biofilm dispersion (Barraud et al. 2009). However, all these signalling molecules can only influence bacteria in their vegetative state. When they are dormant, they cannot respond to the signals. And it is a fact that in biofilms, a large proportion of the cells is in a resting, dormant or viable but nonculturable (VBNC) state and not affected by these molecules. Furthermore, none of them eradicates existing even fully viable biofilms completely. Therefore, their use as antifoulants in technical systems appears very limited, particularly if one considers that it is not easy and by no means cheap to produce them in the required amounts and to apply them in the required sites. Due to their biological nature, they have limited life spans and may be degraded before they have completed their function. It is the great variability of EPS, which protects biofilms and, in turn, limits the success of enzymatic antifouling strategies.

4.5   Surface Monitoring

A big problem for the implementation of timely countermeasures is the already mentioned fact that the surfaces of technical systems usually are poorly or not at all accessible. The response in practice is frequently preventive overdosing of cleaning and biocidal chemicals, which in turn can damage industrial equipment. Also, an environmental burden is generated when the employed substances are released into wastewater, which will have to undergo specific treatment for removal of these substances. Efficiency control of cleaning is usually performed in the reverse way as biofouling is detected: by improvement of process parameters or product quality. This means that it is very indirect and vague and can easily lead into a new round in the vicious circle as depicted in Fig. 2.

Conventional monitoring methods employ sampling of defined surface areas or on exposure of test surfaces ("coupons") with subsequent analysis in the laboratory. A classical example is the "Robbins device" (Ruseska et al. 1982), which consists of plugs smoothly inserted into pipe walls, experiencing the same shear stress as the wall itself. After given periods of time, they are removed and analyzed in the

laboratory for all biofilm-relevant parameters. The disadvantage of such systems is the time lag between analysis and result.

What is lacking is information about site and extent of fouling deposit and, thus, effective and down-to-the-point countermeasures. Therefore, it would be very useful to have "eyes in the system," which allow for the detection of biofilm growth before it turns into biofouling. This is the case for advanced surface monitoring (Flemming 2003; Janknecht and Melo 2003). Systems are needed which have to provide information about the presence of a deposit, its quantity, thickness and distribution, the nature and composition of the deposit, and the kinetics of formation and removal to assess the fouling potential and cleaning success. Some criteria and characteristics for early warning systems are:

– Continuous and automated detection of fouling-relevant parameters;
– Reliable and fast response;
– Easy handling combined with little maintenance;
– Feasible in controlled conditions in the laboratory and in the field situation;
– Easy to interpret output software.

This information should be available online, *in situ*, in real-time, nondestructively, and suitable for data processing and automatization of response.

Such systems will be mainly based on physical principles, some of which have been addressed earlier (Nivens et al. 1995). To meet the demands as listed above, a technique must

– Function in an aqueous system;
– Not require sample removal;
– Provide real-time data;
– Be specific for the surface, that is minimize signal from organisms or contaminants in the water phase.

As early as 1985, Hillman and Anson gave the most comprehensive overview until today on physical measuring principles related to fouling, which meet quite a few of requirements above. Strangely, almost none of them made it to the market, although they were very sophisticated and original. They included already changes in ultrasound, heat transfer resistance, and Nivens et al. (1995) have given an excellent overview on continuous nondestructive biofilm monitoring techniques, including Fourier transform-infrared (FT-IR) spectroscopy, microscopic, electrochemical and piezoelectric techniques. An important aspect has to be taken into account: physical methods mostly respond to nonspecific effects of biofilms. Three levels of information provided by these methods can be identified (Flemming 2003):

4.5.1   Level 1 Monitoring

Level 1 monitoring devices can be classified as systems which detect the kinetics of deposition of material and changes of thickness of deposit layer but cannot differentiate between microorganisms and abiotic deposit components.

They provide information nondestructively, on line, *in situ*, continuously, in real time, and with a realistic potential as a signal for automatic countermeasures. Some examples of this category have already been successfully applied to biofilms (Table 3). It is surprising that they are only very reluctantly accepted in the market an developed further to practical application, although they might save considerable values – blind dumping of biocides still remains the common practice.

An interesting approach for biofouling monitoring in separation membranes has been developed by Vrouwenvelder et al. (2006), using a membrane fouling simulator. This device both determines friction resistance and allows for optical inspection of the surfaces. In an exciting study, it was combined with NMR imaging, revealing the actual hydrodynamics in the device and the dominant role of the spacer for biofouling in membrane modules (Von der Schulenburg et al. 2008).

Table 3 Examples of level 1 monitoring devices

Device	Principle	References
Rotoscope	Determination of light absorption in response to deposit formation	Cloete and Maluleke (2005)
Differential turbidity measurement (DTM)	Two turbidity measurement devices, one of them cleaned, determination of difference between both caused by surface deposit on measurement window	Klahre and Flemming (2000) and Wetegrove and Banks (1993)
Hot wire, heat transfer resistance	Determination of heat transfer changes in response to deposit	Hillman and Anson (1985), Fillaudeau (2003)
Fiberoptical device (FOS)	Determination of light backscattered from deposit on top of a light fiber	Tamachkiarow and Flemming (2003)
Electrochemical measurement device	Determination of changes in electric conductivity caused by material deposited on the surface	Nivens et al. (1995), Bruijs et al. (2000), Mollica and Cristiani (2003)
Acoustic fouling detector	Acoustic backscattering by deposit	Hillman and Anson (1985)
Quartz crystal microbalance	Quenching of frequency of quartz crystal by deposit	Nivens et al. (1995), White et al. (1996), Helle et al. (2000)
Mechatronic surface sensor	Sonic actuator and detector on surface, determination of vibration response	Pereira et al. (2007)
Surface acoustic waves	Determination of difference between speed of acoustic waves with and without deposit	Ballantine and Wohltjen (1989)
Photoacoustic spectroscopy sensor	Absorption of eletromagnetic radiation inside a sample, where nonradiative relaxation processes convert the absorbed energy into heat. Due to thermal expansion of medium, a pressure wave is generated, which can be detected by microphones or piezoelectronic transducers	Schmid et al. (2003, 2004)
Friction resistance measurement	Determination of pressure drop due to biofilm roughness	Hillman and Anson 1985, Eguia et al. (2008)

4.5.2 Level 2 Monitoring

In level 2, systems can be categorized, which can distinguish between biotic and abiotic components of a given deposit. A suitable way to accomplish this is the specific detection of signals of biomolecules. Examples are:

Use of autofluorescence of biomolecules such as amino acids, for example, tryptophane or other biomolecules (Angell et al. 1993; Nivens et al. 1995; Zinn et al. 1999; Kerr et al. 1998; Wetegrove 1998). Such molecules are considered as representative for the presence of biological material. Again, this is only true for systems which normally do not contain biomass, for example, heat exchangers, membrane systems for water treatment, or process water systems. However, it seems that the discrimination of the fluorescence signals of such molecules is difficult to identify, in particular in presence of quenching substances.

FTIR-ATR-spectroscopy specific for amid bands. This approach is suitable for systems, which usually do not contain biological molecules, for example, cooling or process water systems. One way to follow this approach is a bypass pipe with IR transparent windows. For measurement, the water is drained transiently and the measurement is performed (White et al. 1996; Flemming et al. 1998). A very elegant system has been developed by Wetegrove and Banks (1993) and is called the "rotating disk device." This device is based on a disc which is mounted eccentrically on an axis. The lower part is immersed into a water system. After given intervals, the disk turns upside and is analyzed by IR spectroscopy. Strictly spoken, these systems are not completely continuous but they still fulfil fundamental demands of monitoring systems.

Microscopical observation of biofilm formation in a bypass flow chamber and morphological identification of microorganisms (Nivens et al. 1995). Microscopically, however, it may be difficult to distinguish microorganisms from agglomerated abiotic material without application of a dye. Also, microscopic observation requires either a microscopist who more or less continuously carries out the work or a powerful image analysis system, which encounters the same problems as the microscopist when complex deposits accumulate.

4.5.3 Level 3 Monitoring

Systems, which provide detailed information about the chemical composition of the deposit or directly address microorganisms. An example:

FTIR-ATR-spectroscopy in a flow-through cell. In such an approach, not only the amide bands are considered but also the entire spectrum of medium infra red, which has proven to be the most indicative for biological material. The system is composed of an IR-transparent crystal of zinc selenide or germanium which is fixed in a flow-through cell (White et al. 1996; Flemming et al. 1998). The attenuated total reflection (ATR) spectroscopy mode allows to specifically receive the signals of material depositing on the ATR crystal because the IR beam penetrates the medium it is embedded in, that is, water, only into a maximum depth of 1–2 μm. Such systems

allow to distinguish and identify abiotic and biotic material, which attaches to the crystal surface. Raman spectroscopy and microscopy may also provide such information (Wagner et al. 2009).

NMR imaging of deposits in pipes or porous media. Nuclear magnetic resonance imaging (NMRI) techniques were employed to identify and selectively image biofilms growing in aqueous systems (Hoskins et al. 1999). This technique can give information about the extent and spatial distribution of the biofilm and/or deposit and information about the chemical nature of certain components.

5 Conclusions

From the considerations as outlined in this review, it is obvious that successful strategies against biofouling should be based on integrated approaches, which consider the entire system to be protected. It is not possible to eradicate biofilms once and forever. Requests like this from practice remind to the well-known situation when children hate to brush their teeth. Until now, there is no way to brush teeth once and forever. If this is acknowledged, it is possible to learn how to live with biofilms and minimize biofouling problems – which requires just some attention.

The most promising approaches include technical hygiene ("good housekeeping") to minimize the fouling potential of the water phase, for example, by keeping bacterial numbers as well as nutrients as low as possible. Easy-to-clean surfaces and materials which do not support microbial adhesion and growth are another element of integrated antifouling strategies; here, one can learn a lot from food industry, in particular, the implementation and application of the HACCP concept (hazard analysis critical control point; Mortimore, 2001). That includes cleaning-friendly design of the systems and material surfaces, which are smooth and do not leach biodegradable substances such as plasticizers and other additives to plastics.

Establishment of early warning capacity is important to initiate timely countermeasures. Surface monitoring will help a lot, either performed by regular sampling of accessible surfaces or by following fouling-related parameters using
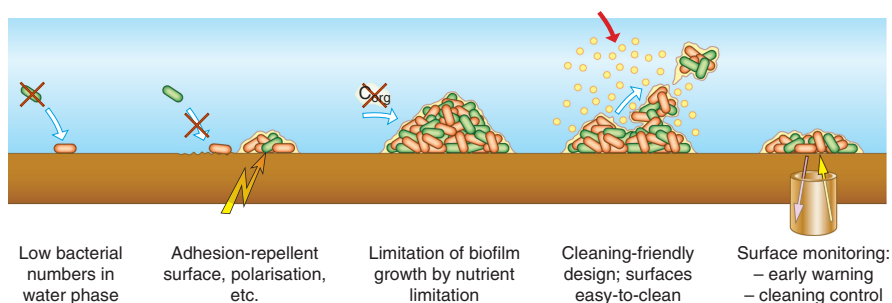

| Low bacterial numbers in water phase | Adhesion-repellent surface, polarisation, etc. | Limitation of biofilm growth by nutrient limitation | Cleaning-friendly design; surfaces easy-to-clean | Surface monitoring: – early warning – cleaning control |

Fig. 8 Key elements of an integrated antifouling strategy

devices as presented earlier. Integrated approaches are, in a nutshell, depicted in Fig. 8:

Practically, all components required for integrated solutions are already available and only have to be assembled and adapted to their particular application. However, such solutions require a shift of paradigms and of the point of view, away from the so much desired one-shot solutions. Tailored solutions can be applied right now and only have to be selected and adapted from the big range of tools, many of which have been mentioned in this review. And although optimal solutions not always exist, the already present ones would proof very effective if applied in the context of holistic approaches. This is where further research should be dedicated – in particular, for longer-term, sustainable solutions. The benefit would be much more success in antifouling and much less environmental damage by biocides, disinfectants and other components, which we do not want to further pollute our waters.

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