Role and levels of real-time monitoring for successful anti-fouling strategies – an overview

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Abstract Biofouling is a biofilm problem and any anti-fouling strategy will be greatly improved if the site and extent of biofilm growth can be monitored. A suitable monitoring system will provide early warning capacity and allow for specific optimization of countermeasures. As water samples do not give reliable information about biofilms, surface sampling is mandatory. Conventional biofilm monitoring techniques rely on removal of material from representative sites or on analysis of test surfaces which have been exposed. This procedure is time consuming and, depending on the parameters to be measured, requires skilled laboratory personnel. There is a strong demand for direct, on-line, in situ, continuous, non-destructive real-time information about biofilms in a system. Such demands can only be fulfilled by physical or physico-chemical methods, a number of which have already been successfully applied for biofilm monitoring. It is important, however, to be aware of the actual parameter they refer to in order to interpret the data properly. Three levels of information can be identified: (i) systems which detect increase and decrease of material accumulating on a surface but cannot differentiate between biomass and other components of a deposit, (ii) systems which provide biological information and distinguish between biotic and abiotic material, and (iii) systems which provide detailed chemical information. Examples for all three levels are presented and discussed.

Keywords Anti-fouling strategy; biofilms; biofouling; real-time monitoring

Introduction
Biofouling is the undesired deposition and growth of microorganisms on surfaces such as heat exchangers, ship hulls, drinking and process water treatment, storage and distribution systems or other technical environments, causing significant economical losses (Flemming, 1996, 2002a,b). Biofouling has been clearly identified as a biofilm problem. Biofilm development in technical systems cannot be avoided as they are operated under sterile conditions (Flemming et al., 1993). Adhesion to surfaces and subsequent biofilm growth belongs to the genuine microbial survival strategies. The impact of biofilms varies in technical systems, thus, they can tolerate biofilms to a lesser or greater extent until an interference of process or product quality is observed, depending on individual requirements and process sensitivity. In order to keep biofilm growth below a given “threshold of interference” (Flemming, 1996), it is necessary to obtain information about the actual extent of biofilm growth for timely and effective countermeasures (Griebe and Flemming, 1998). Such a “threshold of interference” will vary according to the demands of a given process and may vastly change between a paper mill and a purified water system. No system, however, will be operated completely sterile and, thus, will inevitably contain biofilms (Figure 1).

Problems in conventional anti-fouling strategies
Conventional countermeasures against biofouling are hampered in practice by five expensive mistakes which are identified as follows.
1. Biofilm monitoring is performed by process performance or product quality – no early warning system. Thus, biofouling is detected only in a very late phase, when it has already started to hurt. This is the most expensive way to detect problems because it not
only costs in terms of process performance or product quality but also, because countermeasures are less efficient the later they are taken.

2. Detection biofouling is performed in many cases by exclusion of other possible causes for problems, thus, indirectly. If no physical, chemical or technical explanation is valid, biofouling is suspected. This is practical experience from many biofouling cases. If eventually biofouling is considered, usually, samples are taken from the water phase. However, such samples contain microorganisms which have either grown in the water phase or have been detached from biofilms. As detachment occurs randomly, there is no reliable correlation between cell numbers in the water phase and the extent of biofilm growth. Also, microbiological water phase data do not provide information about the location of biofilms.

3. Disinfection is misunderstood as cleaning. When the problem is identified and localized, the usual remedial approach is analogous to that in medicine: biofouling is treated as a “disease” of the plant by microbial infection, thus, killing the organisms will cure the disease. However, while living organisms have systems to dispose of dead microorganisms, technical systems do not. Thus, “disinfection” will leave dead biomass where it had accumulated during operation. In many cases, the biomass itself is the problem, e.g., when biofilms interfere with heat exchange, hydraulic resistance of membranes or friction resistance, the problem will not be solved. Some oxidizing disinfectants may cleave the bonds between the extracellular polymeric substances (EPS) which are responsible for coherence of the biomass. However, practical experience shows that this effect is rarely sufficient to remove the biomass. Rapid regrowth is to be expected as biofilm organisms are particularly resistant to biocides (LeChevallier et al., 1988) and survivors will rapidly regrow at the expense of dead biomass. In such cases, a “saw tooth curve” with an increasing base line is frequently observed in practice.

4. Nutrients are not limited. It must be taken into consideration that in a non-sterile system microorganisms are always present, are waiting for nutrients. Thus, biodegradable substances must be considered as potential biomass. Disinfection measures, however, rarely also reduce the concentration of nutrients. Lechevallier (1991) showed that the amount of assimilable organic carbon can rise by the use of ozone if recalcitrant substances are present which are partially oxidized and made better biodegradable.
5. Assessment of the efficacy of countermeasures is performed by process performance or product quality, not by analysis of the biomass remaining on surfaces which represents further biofouling potential. This approach provides no early warning capacity and frequently leads to high preventive overdosage of biocides which may harm the system and will create problems when then have to be disposed into the waste water.

Avoiding mistakes 1–3 and 5 would require biofilm monitoring systems which provide information about the extent of biomass accumulated on surfaces. Therefore, quantitative information about deposits on surfaces is highly desirable. This demand will be increased by the new biocide guideline of the European Commission (EC) which will restrict the application of biocides drastically. Optimization of biocide and cleaner application requires more sophisticated approaches and techniques.

A possible option can be “Biofilm management” (Flemming, 2002a,b). The approach principally accepts the occurrence of biofilms and aims to keep their development below a threshold of interference which has to be defined for a given system to be protected. In the first place, that strategy includes a fouling factor analysis, in particular addressing nutrients in the water phase as potential biofouling mass. The main components of such a strategy are:

(i) nutrient limitation as nutrients must be considered as potential biomass:
(ii) monitoring of biofilm development and location. The term “monitoring” is used here as a synonym for observation of a parameter over time

These considerations make it clear how crucial monitoring of biofilm development is for effective biofilm management. This allows for timely cleaning measures and optimization of their efficacy. However, such requirements meet a general analytical problem, as analytical chemistry and microbiology can detect very small amounts of chemicals or microorganisms in a volume, e.g., water or air, but as soon as material is deposited on a surface, in most cases it is necessary to remove the deposit and analyze it in the laboratory. Thus, conventional monitoring systems are mostly based on test surfaces, so-called “coupons” of materials exposed and removed after a given period of time and subsequently investigated by microbiological and biochemical methods. This approach provides results only after a relatively long time and usually requires sophisticated laboratory equipment and skills.

Early warning systems are needed which have to provide information about the nature of a deposit, its quantity, thickness and distribution and the kinetics of formation and removal in order to assess the fouling potential. This information should be available on line, in situ, in real time, non-destructively and suitable for computer-aided automatization – without requiring sample removal, staining or other secondary procedures. Such systems will mainly be based on physical principles, some of which have been addressed earlier (Nivens et al., 1995). In order 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, i.e., minimize signal from organisms or contaminants in the water phase

Levels of information provided by monitoring systems

In technical systems, pure biofilms are rare. In most cases, deposits develop which consist of biomass which includes organisms and their matrix of extracellular polymeric substances (Flemming and Wingender, 2001), adsorbed particles and precipitated material. Thus, mixed deposits have to be expected which require combined and differentiated countermeasures.

Three levels of information can be clearly distinguished, in which current monitoring devices can be distinguished.
Level 1 monitoring devices

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 non-destructively, 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. They can be distinguished as either local or integral sensors. Examples for local sensors are given below.

**Fiber optical device (FOS)** consisting of optical fibers, penetrating the walls of water pipes with the tip even to the inner pipe surface, using the intensity of backscattered light for assessing the thickness of the deposit which has accumulated on the tip of the fiber (Tamachk iarow and Flemming, 2003).

**Differential turbidity measurement device (DTM)**, which consists of two turbidity measurement devices, one of them being continuously cleaned. The difference between the cleaned and non-cleaned device is caused by the deposit; cleaning can be performed by water jet (Klahre and Flemming, 2000) or by mechanical wiping (Wetegrove and Banks, 1993a).

**Acoustic fouling detector**, based on the measurement of acoustic scattering cross-section acoustic impedance of a biofouling layer (Hillman and Anson, 1985).

**Quartz crystal microbalance device (QMB)**, exploiting the decrease of vibration caused by material deposited on the crystal surface (Nivens et al., 1995; White et al., 1996). Earlier versions of these systems were extremely sensitive to temperature changes of the water while more recent devices have addressed this problem successfully (Helle et al., 2000).

**Electrochemical measurement devices** which are based on the change in electric conductivity of a surface caused by a deposit (Nivens et al., 1995), on cathodic depolarization which is indirectly attributed to microorganisms (Licina and Nekoksa, 1993; Bruijs et al., 2001; Mollica and Cristiani, 2003).

**Redox electrode**, integrated in the surface on which a biofilm growth and measuring the redox potential generated by the activity of biofilm organisms (Holtmann and Sell, 2002). The signal, however, may also be generated by abiotic processes.

**Photoacoustic spectroscopy sensor (PAS)** which is based on the absorption of electromagnetic radiation inside a sample where non-radiative relaxation processes convert the absorbed energy into heat. Due to the thermal expansion of the medium, a pressure wave is generated which can be detected by microphones or piezoelectronic transducers (Schmid et al., 2003).

Examples for sensors of level 1 which integrate over a larger distance or area are:

**Surface acoustical waves (SAW)**, determining the difference of the speed of surface waves on surfaces with and without deposits (Ballantine and Wohltjen, 1989).

**Friction resistance measurement**, exploiting the pressure drop which is caused by increasing thickness and roughness of a given deposit (Roe et al., 1994; Lee et al., 1998).

**Heat transfer exchange resistance device**, based on the decrease of the heat transfer rate by
fouling layers, e.g.: “hot wire method”, this is an experimental device developed to measure thermal resistance of corrosion products. Small wire immersed in fluid and heated electrically. Degradation of temperature of fluid measured as products (which can also be biofilms) built up on wire (Hillman and Anson, 1985).

All these methods have in common, that they respond to material which is deposited on the surface under observation. Some of them respond to changes which can be related to microbial activity such as the electrochemical devices. If the signals are attributed to biofilms, independent methods are necessary in order to confirm this. Otherwise, deposited materials or chemical reactions other than of microbial origin may cause wrong interpretations. However, the value of the information as provided by such sensors should not be underestimated although it will indicate biofilms only indirectly – it has early warning capacity and allows for timely countermeasures. Interestingly, many of the above listed systems are proven to be effective but have not made their way into wider practical application. This may be attributed to a market which is not fully aware of the values which can be saved by good monitoring.

**Level 2 monitoring devices**

*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 given below.

**FTIR-ATR-spectroscopy specific for amide bands.** This approach is suitable for systems which usually do not contain biological molecules, e.g., 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). An very elegant system has been developed by Wethergrove and Banks (1993b) and is called the “rotating disk device”. This device is based on a disc which is mounted excentrically on an axis. The lower part is immersed into a water system. After given intervals, the disk turns upside and is analyzed by infrared spectroscopy. Strictly spoken, these systems are not completely continuous but they still fulfill fundamental demands of monitoring systems.

**Use of auto-fluorescence** of biomolecules such as amino acids, e.g., 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, e.g., 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 the presence of quenching substances.

**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, microscopical 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.

**Level 3 monitoring devices**

Systems which provide *detailed information about the chemical composition* of the deposit or directly address microorganisms. An example is given below.
**FTIR-ATR-spectroscopy in a flow-through cell.** In such an approach, not only the amide bands are considered but 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 one to specifically receive the signals of material depositing on the ATR crystal because the IR beam penetrates the medium it is embedded in, i.e., water, only into a maximum depth of 1–2 µm. Such systems allow one to distinguish and identify abiotic and biotic material which attaches to the crystal surface.

A fourth level could be identified which would allow for discrimination of living from dead microorganisms on a surface. However, there is no approach in sight which could meet this demand.

A particular problem is the mutual comparison of the various methods. Roe et al. (1994) have integrated an annular reactor (“RotoTorque”) in which biofilm accumulation was determined by light transmission, a tube system for measurement of pressure drop, a heat transfer resistance measurement device and a coupon system for direct biofilm measurement in a recycle loop system. The Torque, while showing good sensitivity to biofouling and close tracking in duplicate monitors, exhibited somewhat poorer correlation with mass accumulation and with secondary fouling parameters such as heat transfer resistance and pressure drop. Light transmittance through biofilm in the annular reactor was also a sensitive measure of biofouling, but, being a localized measurement, it demonstrated considerably more variability than the other biofouling probes. In general, the correlation between biofilm accumulation and the signals of the various sensor systems did not correlate very well. However, if in a given system a sensor provides information about increasing or decreasing deposits, this would be a helpful component in an anti-fouling strategy.

**Discussion**

This list of devices as given above is far from being complete. Interestingly, most of the approaches have never exceeded successful proof of operation at laboratory level. Obviously, industry considers optimisation of anti-fouling measures by early warning systems and by assessment of success as superfluous. Thus, preventive over- and/or underdosing of biocides is the actual practice and the frequent lack of success is the cause of substantial losses.

It seems to be suitable to develop a “tool box” with different monitoring devices, suitable for different levels of fouling layer thickness as well as of accuracy and information. The methods reported in this paper are part of this concept, which is crucial in order to develop anti-fouling strategies which are adapted to areas which range in their fouling potential from ultrapure water biofouling to paper production.

Quite a number of sensors have been published and patented which come close to the requirements as discussed earlier which qualify them for an advanced anti-fouling strategy. Interestingly, many of these sensors are either locally immobilized, delivering information from one site which is taken as representative for a larger surface area or give an integrated signal, such as provided by measurement of friction or heat transfer resistance. However, sensors which allow for scanning of surfaces on demand would be very feasible for assessment of cleanliness of, e.g., food surfaces, but there is no information available about the successful development of such sensors. It is surprising that so many intelligent, reasonable, and in principle simple solutions for biofilm monitoring have been developed but have never moved beyond laboratory status. Still, contemporary anti-fouling strategies refrain from direct information about the situation on surfaces and, instead, blindly apply biocides. It must be clearly stated that this is a waste which could be circumvented by using...
proper monitoring devices fulfilling the requirements as identified earlier. It is only a matter of time until biofilm monitoring will be a state-of-the-art technique, using many different approaches, of which quite a few may be on the lists as presented above.

References


