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ENVIRONMENTALLY ACCEPTABLE CONTROL OF MICROBIAL BIOFILMS

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ABSTRACT

Surfaces that are exposed to liquids in the environment become covered by microorganisms at a rapid rate. Under good growth conditions these cells divide and form a layer of cells called a biofilm. This chapter will give a survey of the methods that are used to date to prevent the formation of biofilms (biofouling), and consider what kind of approaches might be used for future protection due to their environmental friendliness.

DEFINITION OF BIOFOULING

The undesired deposition of cells and the subsequent formation of a cell layer (biofilm) on a surface are called biofouling (Flemming *et al.*, 1996). Biofouling occurs between liquid-solid, gas-solid or even liquid-liquid interfaces. The largest problems with biofilm growth are encountered at the liquid-solid interface. Thus, discussion will be limited to this particular interaction.

PROBLEMS CAUSED BY BIOFOULING

Biofilms contribute to a range of costly problems in daily life. They may be responsible for the biodeterioration of materials, e.g. corrosion of metals (Ford and Mitchell, 1990; Little *et al.*, 1991), degradation of polyester-polyurethane (Gu *et al.*, 1998b), and deterioration of concrete (Dierks *et al.*, 1991; Gu *et al.*, 1998a). Water treatment plants are especially sensitive to biofouling (Byrd *et al.*, 1991; Block *et al.*, 1993; van der Wende *et al.*, 1989; Speth *et al.*, 1998; Donlan, 1999) because a continuously high quality of the drinking water has to be maintained. However, limited biofouling is often observed in drinking water distribution systems (Camper *et al.*, 1999) and can reduce the transport of freshwater through tubes (Munson *et al.*, 1990; Lewandowski and Stoodley, 1995). Analogous observations were reported for biofouling of ship hulls; biofilms increase the drag of ships (Bohlander, 1991) and therefore significantly raise the consumption of fuel (Alberte *et al.*, 1992). Biofilms also may impose an imminent danger or even a life threatening problem to people that

When cells attach successfully to a surface, genes are activated which lead to the synthesis of various compounds (Angles and Goodman, 1999). An important category for the biofilm formation represents the extracellular polymeric substances (EPSs), which consist of proteins (adhesins) and extracellular polysaccharides (Sutherland, 1980; Allison and Sutherland, 1987; Davies *et al.*, 1993) and are typically found in biofilms at concentrations of 1-2% w/v (Christensen and Characklis, 1990). Polysaccharides are typically composed of repeating sugar units, usually glucose, galactose, mannose, rhamnose, N-acetylglucosamine, glucuronic acid and galacturonic acid. They are assembled intracellularly into a polymer from sugar-nucleotides (e.g. UDP-galacturonic acid) via lipid-linked intermediates (Sutherland, 1982). Three basic types of extracellular polysaccharides are observed: lipopolysaccharides (LPSs), capsular polysaccharides (CPSs) both located on the outer membrane of Gram-negative cells (Whitfield and Valvano, 1993), and slime polysaccharides (SPSs). SPSs are completely released from the cell and differ from LPSs and CPSs with respect to molecular structure and sugar composition (Hughes, 1995). CPSs and SPSs often have extremely high molecular weights, often in the range of millions. Aqueous solutions of SPS are viscous and behave non-Newtonion viz. the viscosity is dependent on the shear rate. The presence of uronic acid and pyruvate in the polymer influences the physical properties significantly, since charged polymers are more soluble in water (Hart *et al.*, 1999) and can react as cation exchangers (Christensen and Characklis,1990; Linton *et al.*, 1999). Thus, in special cases the exopolysaccharides can also take an important role in corroding the surface that the bacterium is attached to. For instance, it was found that *Thiobacillus ferrooxidans* is not able to leach non-ferrous sulphide (synthetic covellite, CuS) when the exopolysaccharides were removed (Pogliani and Donati, 1999). However, the

In the last stage of biofilm formation successfully attached cells further divide and form microcolonies with a complex structure (Lewandowsky, 1999). Allison and Sutherland (1987) concluded that without the synthesis of EPSs no microcolonies were formed. An indirect confirmation of this observation was only reported recently: biofilms that were exposed simultaneously to low nutrient concentrations, high flow rates (up to 0.72 m s^{-1}), and turbulent flow conditions ($4200 < Re < 12000$) showed an increased physical stability (Melo and Vieira, 1999). Interestingly, the substrate consumption flux at high flow rates (0.62 m s^{-1}) was calculated to be considerably smaller than at slower flow (0.28 m s^{-1}). Further investigations revealed that biofilm structure is highly heterogeneous and reaches after a certain time a constant thickness due to biomass loss and cell growth (van Losdrecht *et al.*, 1995; Huang *et al.*, 1998; Xu *et al.*, 1998). Consequently, biofilm growth and steady-state conditions are difficult to describe mathematically (Wanner, 1996).

The system seems to be more complicated because it has been reported that cells in biofilms are able to communicate (Davies *et al.*, 1998) and are able to exchange genes (Angles and Goodman, 1999; Helmström and Kjelleberg, 1999). Moreover, a mixed culture of *Enterobacter agglomerans* and *Klebsiella pneumoniae G1* showed a more successful formation of biofilm than in isolation (Skillman *et al.*, 1999). This was explained by the affinity of the EPSs of both strains: a 2:1 mixture of *Enterobacter*-EPS : *Klebsiella*-EPS reached a higher viscosity than with a 1.1 mixture.

To date, the strategy of controlling heavily fouled surfaces is to use strong chemicals to kill the microorganisms. However, biofilms are more difficult to remove than previously thought (Muraca *et al.*, 1990; Callow, 1993; Williams *et al.*, 1997). Extensive research has revealed that cells of a biofilm are generally more resistant to

Depending on the size of the system, frequent biocide additions may become a very expensive way to control biofilms. First, the costs of the required chemicals may be large over time. Usually expensive oxidizing (bromine, chlorine, iodine, peracetic acid, hydrogen peroxide) and non-oxidizing agents (benzoate, bisulfite, formaldehyde, glutaraldehyde, quaternary amines) are applied in various processes. Second, without some mechanism for monitoring biofilm thickness or formation rates, the frequency of biocide addition is difficult to titrate for maximum effectiveness. Over-dosing can waste costly biocides while under-dosing may not provide effective control. Third, oxidizing biocides may have an additional negative side effect, viz. the housing of the system may corrode. This is an important problem frequently encountered in drinking water distribution systems. Electrochemical reactions at the pipe surface may cause the formation of pits which can originate larger nodules composed of ferric hydroxide. Growth of the corrosion nodules increases turbulent resistance to flow within the pipe and reduces the shear stress on the surface. The increase in turbulent boundary layer favors the adhesion of nutrients to the biofilm, improving the nutrition of the habitat for microbial cells. Recently, it has been shown with glass capillary flow cells that cell growth under such turbulent flow conditions was not as fast as under laminar flow conditions. However, a denser packing and higher cell numbers were detected when steady-states were obtained (Stoodley *et al.*, 1999). This confirms the observation that samples of iron tubercles in drinking water distribution pipes showed higher counts of coliform bacteria than not corroded systems (LeChevallier *et al.*, 1987).

Once a biofilm is formed, removal requires increased biocide concentrations (Brown and Gauthier, 1993; Chen *et al.*, 1993; Xu *et al.*, 1996).

The research on antifoulants needs accurate and appropriate test systems. Ludensky (1998) suggested a method that enables the on-line monitoring of the antifoulant efficiency for a heat exchanger. Parameters such as heat transfer, dissolved oxygen, and the pH of the solution were measured continuously, enabling the documentation of the efficiency of biocides.

Open Systems

The treatment of biofilms is much more difficult in open systems than in closed or semi-open systems. Surfaces exposed to seawater or lake water are quickly covered by a bacterial layer (Kerr *et al.*, 1998). This may enhance the attachment and fouling by larger organisms such as mussels and algae (Evans, 1981; Kirchman *et al.*, 1982; Holmström and Kjelleberg, 1994; Gu *et al.*, 1997). Thus, the best approach is to reduce the formation of bacterial biofilms as much as possible. As previously mentioned, this can be performed through frequent and consequently expensive, cleaning of the surface. This is usually done with water jets (Swain and Schultz, 1996), steam (Flemming *et al.*, 1996), ultrasound (Zips *et al.*, 1990) or acid and base baths (Speth, *et al.*, 1998). A frequently encountered problem with these methods is that the surface may be harmed by strong chemicals and treatment. The incomplete removal of organic compounds such as exopolysaccharides may propagate new fouling. In addition, these methods are labor intense and time consuming and may not be applicable to all fouling prone systems, e.g. large ships and oilrigs.

As previously mentioned, researchers have concluded that the avoidance of biofilm has to occur at the very beginning, with reduction of microbial attachment (Gerhardt *et al.*, 1988; Holmström and Kjelleberg, 1994). This seems reasonable since

of phase in response to a triggering signal, such as temperature, ionic strength, pH, light, or an electrical field (Galaev, 1995). Ista and Lopez (1998) showed a first application in cleaning of fouled surfaces. They used poly(*N*-isopropylarylamide) (PNIPAAM) which has a lower critical solubility temperature of 32°C. Test surfaces were fouled with *Halomonas marina* in natural bay water above 32°C. More than 90% of the attached fouling material could be removed when a phase change in the PNIPAAM was provoked by the temperature decrease. These results indicate that such materials have great potential and more research should be performed in this field.

The construction of an active and biocidal surface is another approach to reducing cell attachment. Toxic metals like copper, zinc, and silver appeared to have an antifouling effect when used as surface material or when integrated into paint. However, this effect is observed for only a short time because a few bacteria are able to overcome the toxicity by the production of protective exopolymers (Silver and Misra, 1988; Babycos *et al.*, 1993; Geesey, 1994; Rogers *et al.*, 1995; Srivastava *et al.*, 1995; Flemming *et al.*, 1996; Tang and Cooney, 1998). In addition, some surfaces such as copper, rapidly oxidize to insoluble, non-toxic salts. Thus, mechanical cleaning of such surfaces remains a necessity. Depending on the antifouling agents incorporated into the surface coating, cleaning can produce highly toxic waste that must be treated with care, adding to the costs and difficulty of treatment.

The application of DC5700 (3-trimethoxysilyl)-propyloctadecyldimethyl ammonium chloride), a quaternary ammonium compound covalently bound to a silicone matrix, showed promising results (Evans and Clarkson, 1993). However, the application was found to be restricted, since the intramolecular bonding was weakened in presence of sea water. The antifouling effect was lost after 7 d and

with a Robbins device (a flow cell with laminar flow conditions) that TBT-resistant *P. aeruginosa* PAO-1 made up to 50% of a biofilm culture.

The replacement of organotin with high levels of cuprous oxide appeared to reduce biofouling also. The use of such coating material (e.g. ABC^R, Ameron International, Brea, CA, USA) in combination with an anticorrosive coating (epoxy or coal-tar in epoxy) showed acceptable protection for 2 years, but did not attain the initially good performance of organotin (Bohlander, 1997).

Leaching coatings

The diffusion of antifouling compounds from a surface, called leaching, offers another field of applications (Swain and Schultz, 1996). This method of surface protection was copied from nature, since marine organisms like sponges (Thompson, 1985; Thompson *et al.*, 1985; Sears *et al.*, 1990), Gorgonian corals (Keifer *et al.*, 1986; Vrolijk *et al.*, 1990), and the eelgrass *Zostera marina* (Harrison and Chan, 1980; Todd *et al.*, 1993) contain naturally occurring compounds shown to inhibit a wide range of fouling organisms. There are now more than 90 antifouling compounds of natural origin described in literature (see survey by Clare, 1996). The structure of natural antifoulants (see Figure 2) is usually complex and therefore the production by chemical synthesis is in many cases very difficult (Clare, 1998). In most cases, the chemical reaction responsible for the antifouling activity of these compounds is unknown.

INSERT FIGURE 2

MERR for a particular antifoulant can be determined. Typical release rates are summarized in Table 1.

INSERT TABLE 1

Although simple in principle, the MERR system has been difficult to carry out in practice. Mechanical problems have involved membrane clogging, deterioration of soluble agents in the feed tubes and unrealistically high mass flow of solvent at the membrane surface. Thus, while MERR systems may provide general ranges of effective concentrations, they do not simulate the leaching of antifouling agents from hard coatings.

Optimal leaching rates

Antifoulants that are integrated into a coating leach at decreasing flux rates as the reservoir is drained. The dynamics are derived from the Fickian law and can be calculated according to Higuchi (1963):

$$Q = (D(2A_0 - C_S)C_S t)^{1/2}, \quad (1)$$

where Q is the amount of the diffusant released per unit area over time t, A_0 is the initial diffusant concentration in the matrix, C_S is the solubility, and D is the diffusion coefficient of the diffusant out of the matrix. The flux, or release rate F_{dc} is then given as (Weisman *et al.*, 1992):

USA). This would considerably help in making comparisons of different test panels and moreover, biofouling could be detected at a much earlier state, e.g. through the detection of microorganisms and microalgae.

NEW AND ENVIRONMENTALLY FRIENDLY WAYS TO PROTECT SURFACES FROM FOULING

Studies have shown that bacterial biofilms can inhibit the attachment of larvae of marine organisms (Maki *et al.*, 1988; Holmström and Kjelleberg, 1994). Burchard and Sorongon (1998) showed that this is also the case for the interaction between gliding bacteria. The cells were isolated from a marine biofilm and identified as being members of the genus *Cytophaga*. One strain (RB1057) produced an extracellular glycoprotein with a mass of about 60 kDa and this inhibited the other strain (RB1058) from adhering and gliding on substrata. It was found by the same group that this inhibitor was not effective against other aquatic gliding bacteria. However, a modification of the protein might broaden the impact on other species. Ideally, this antifoulant would be placed covalently bound to a suitable matrix.

The screening for potential microorganisms and their products can be carried out with hydrogels (Gatenholm *et al.*, 1995), where the bacteria are embedded into a gel and are able to live and produce the potential antifoulants. Subsequent attachment tests with larvae or microorganisms will indicate which strains produce effective antifoulants. The responsible chemical can then be isolated and integrated into a leaching or eroding coating.

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scales were shaped in clusters and were elliptic crystals, whereas under no current conditions the scale organized in needle-shaped crystals and much more difficult to be removed. Whether their observation was related to the biological activity was not given.

Bioerodible materials are also under investigation for the *in situ* delivery of anti cancer drugs (Heller *et al.*, 1990; Mathiowitz *et al.*, 1997; Egilmez *et al.*, 1998). Here, the erosion of the surface releases the drug that is integrated in the matrix. Successful studies have been reported with poly(3-hydroxybutyrate) (PHB) as a bioerodible matrix (Akhtar *et al.*, 1992; Gopferich, 1996; Pouton and Akhtar, 1996). Further, biodegradable rubber made of poly(3-hydroxalkanoates) (PHA) (de Koning *et al.*, 1994) seems to have potential for use as an environmentally friendly matrix. The antifouling agent could be incorporated by cross-linking with the polymer through irradiation. The antifoulant would then be released through the bioerosion of the matrix. This could theoretically enhance the efficiency of the antifoulant, since it is postulated that growing cells are more sensitive to toxic compounds (Brown *et al.*, 1988; Williams, *et al.*, 1997). However, this has to be confirmed experimentally.

FUTURE CHALLENGES

The reviewed strategies suggest that the inhibition of microbial fouling by highly toxic chemicals is somewhat ambiguous since they may significantly harm organims. Biological methods represent an alternative, since they are, in general, more environmentally friendly due to their biodegradability. However, it is important to note that rate of biodegradation vary significantly among so-called natural antifouling

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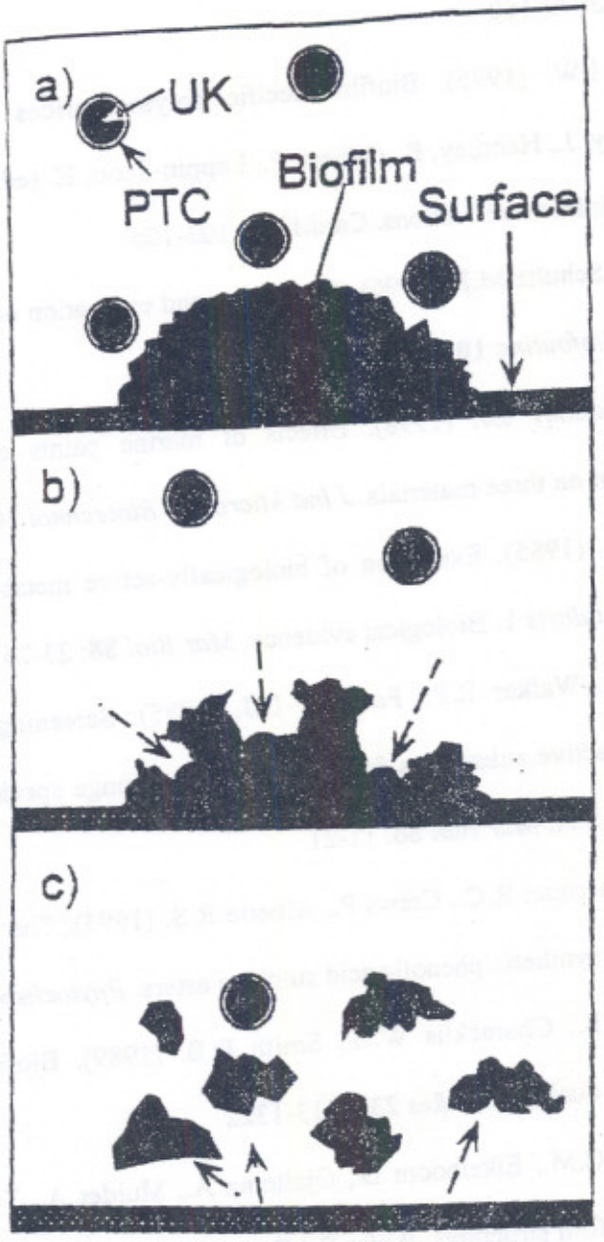
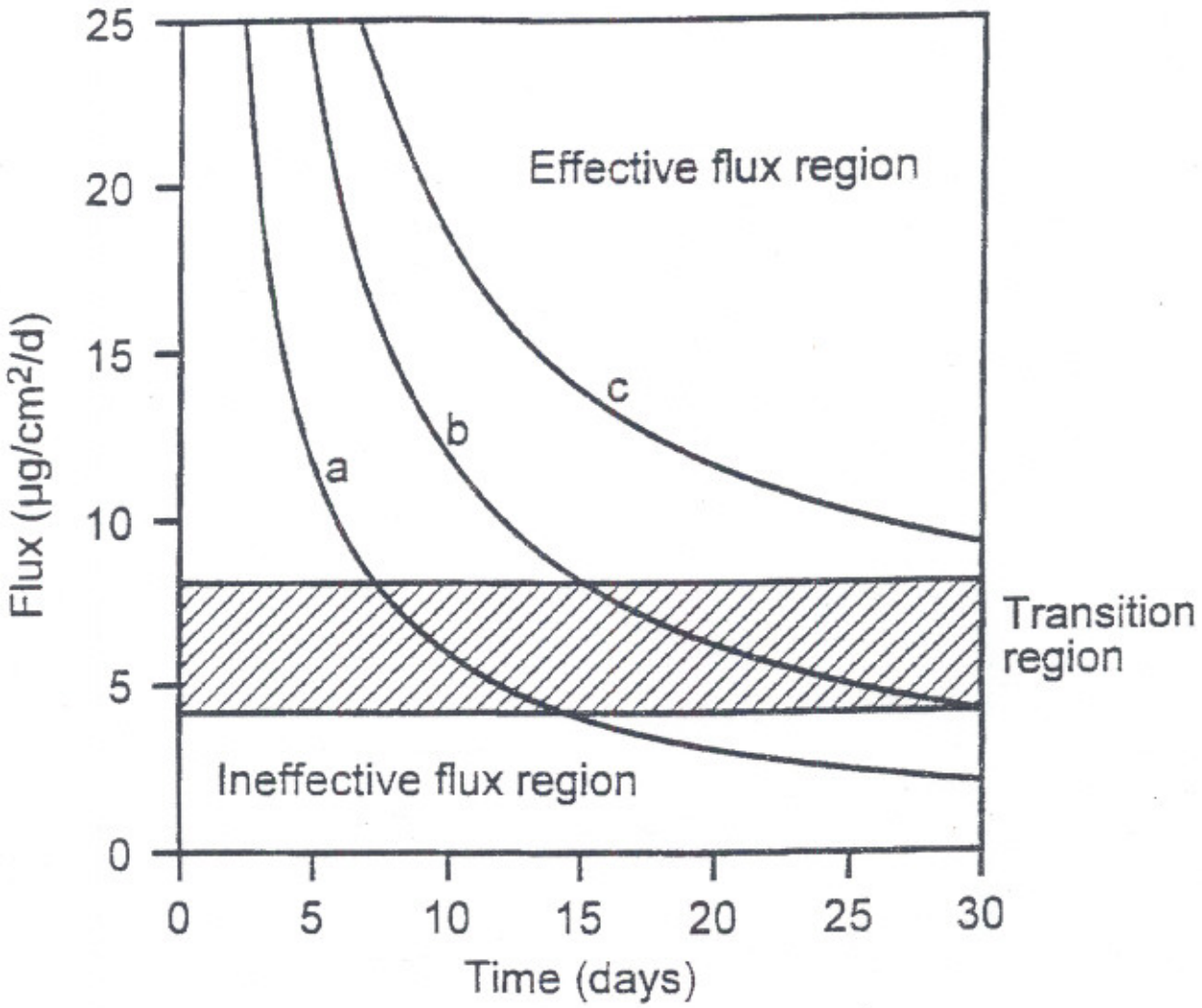

a)
UK
PTC
Biofilm
Surface
b)
c)


25
20
15
10
5
0
Flux (µg/cm²/d)
Effective flux region
a
b
c
Transition region
Ineffective flux region
0
5
10
15
20
25
30
Time (days)