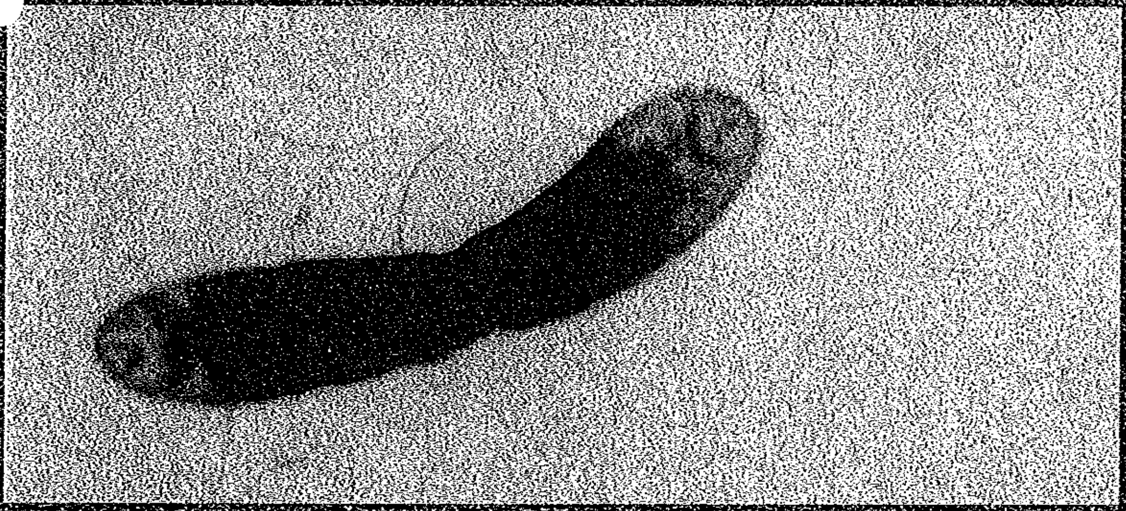
Petroleum Microbiology



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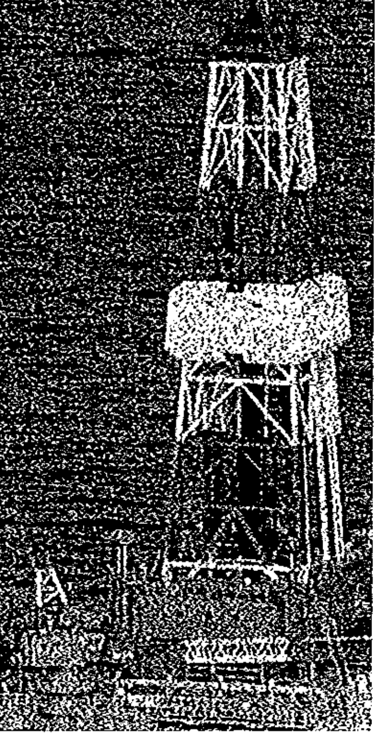

BIOFOULING IN THE OIL INDUSTRY

Peter F. Sanders and Paul J. Sturman

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> ...Tho my teeth are kept usually very clean, nevertheless when I view them in a Magnifying Glass, I find growing between them a little white matter as thick as a wetted flower... I therefore took some of this flower and mixt it... with pure water wherein were no Animals... and then to my great surprise perceived that the aforesaid matter contained very many small living Animals, which moved themselves very extravagantly... The number of these Animals in the scurf of a mans Teeth are so many that I believe they exceed the number of Men in a kingdom. For upon the examination of a small parcel of it, no thicker than a Horse-hair, I found too many living Animals therein, that I guess there might have been 1000 in a quantity of matter no bigger than the 1/100 part of a sand.
>
> *Antonie van Leeuwenhoek (1684), observing a disaggregated dental biofilm*

The attachment and growth of microorganisms on surfaces (the buildup of a slimy biofilm layer generally termed biofouling) are well-established phenomena in many environments and industries. Despite the early recognition of the importance of biofilms and biofouling, serious study only really began in the early 1940s with the pioneering work of ZoBell (1943), who developed the early concepts for the different stages in biofilm development, which lasted for 20 years or more. Thirty years after the work of ZoBell, biologists studying many environmental, medical, agricultural, and industrial systems independently discovered the fundamental importance of biofilm formation; they began to investigate the factors controlling the change from a planktonic growth state to a sessile one and the changes in bacterial metabolism that occur immediately upon attachment of a cell to a surface (Sauer and Camper, 2001). Research on the interaction of bacterial cells with each other, the environment, and the substrate has continued apace since the 1970s (Characklis and Marshall, 1990), with research institutes such as the Center for Biofilm Engineering in Montana (http://www.erc.montana.edu) and societies such as the British Biofilm Club (http://www.biofilmclub.co.uk/) dedicated to the study, control, and use of biofilms. The most recent applications of new techniques in the fields of genetic, biochemical, instrumental, and microscopic analyses have led to a major step forward in our understanding of biofilm processes such as the factors controlling the change from a planktonic mode of growth, the physiological differences between attached and planktonic cells, the detailed structure of biofilms under

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different environmental conditions, and the interspecies and intraspecies interactions that lead to an active and dynamic consortium (Lewandowski, 2000; Geesey, 2001; Stoodley et al., 2002).

Any biofilm from an industrial system will contain a very wide range of aerobic, anaerobic, microaerophilic, heterotrophic, and lithotrophic microorganisms able to grow under an extreme range of environmental conditions of temperature, pressure, pH, salinity, nutrients, toxicity, and water activity (Magot et al., 2000). Biofilm formation causes physical operational problems such as plugging of hydrocarbon reservoir rock, blockage of filters, and flow capacity reduction in pipes. Growth of microorganisms on surfaces is an essential prerequisite for the onset of microbially influenced corrosion and biodeterioration of a wide range of materials. Finally, biofouling can lead to the spoilage of oil field products by degradation, increase in suspended solids, and changes in bulk fluid composition, such as dissolved sulfide generation (Sanders, 2002). Biofouling can, however, be used in a positive way, for example, in the form of microbially enhanced oil recovery (MEOR) techniques and in bioreactors to improve the quality and marketability of the produced hydrocarbon. Understanding and controlling the biofouling process therefore has fundamental significance for the petroleum industry. This chapter describes the evolving models for biofilm development and highlights the role of biofouling in microbially related oil field problems and opportunities covered in other chapters of this book.

BIOFILMS: GENERAL CONCEPTS

In the simplest descriptive terms, a biofilm is a microbial accretion, adherent to a biological or nonbiological surface, and enclosed in an extracellular polymeric matrix of its own production. Biofilms (especially industrial biofilms) may also contain a significant amount of inorganic substances (silt, scale, sand, and corrosion products), entrapped within the extracellular polymeric matrix. Since planktonic marine bacteria were first observed to preferentially attach to available surfaces (ZoBell, 1943), bacterial biofilms have been implicated as the causative agent in a wide range of petroleum production and refining problems. Their ability to thrive over a remarkable range of growth niches, from organic-rich oil-water emulsions to the relatively oligotrophic seawater injection well environment, suggests that bacterial communities can survive in virtually all fluid streams associated with petroleum extraction and processing. An active microbial consortium has, indeed, been confirmed throughout virtually all oil field process systems. Over the past 2 decades, it has become increasingly apparent that biofilms are the preferred mode of growth for most bacteria (Costerton et al., 1978), including those microorganisms of greatest interest to hydrocarbon extraction and petroleum-refining industries.

While early descriptive models of biofilms characterized them as uniformly thick slabs of slime-embedded bacteria (Williamson and McCarty, 1976), our present understanding suggests that mature biofilms are typically highly structured, multispecies microbial communities, encased in a biochemically complex matrix of self-produced extracellular polymeric substances (EPS) (Stoodley et al., 2002). Depending on their composition and activity, biofilms may drastically alter the physical and chemical conditions in their immediate vicinity (Costerton et al., 1994). These changes can lead to many of the common problems associated with biofilm growth, including biofouling, plugging, biologically influenced corrosion, and petroleum product souring (Sanders, 2002).

Biofilms develop in response to both system conditions (external stimuli) and cell-produced chemical signals (internal stimuli). Both these stimuli have significant effects on the structure and activity of biofilms. Biofilms grown under high-shear conditions typically develop a tenacious extracellular matrix and have a relatively thin cross-sectional thickness, whereas those grown in quiescent, nutrient-rich environs are typically thicker (>100 μm in many cases) (Stoodley et al., 1997). These varied biofilm physical properties have a direct impact on the ease of removal by shear or other physical means; biofilms which develop under quiescent conditions tend to be less adherent and are thus easier to remove (Stoodley et al., 2001). Differences in biofilm growth conditions can also lead to heterogeneities that influence the movement of dissolved chemical species (including antimicrobial agents) into and through the biofouling layer, significantly impacting on the effectiveness of any biofilm control program in oil field systems (Gardner and Stewart, 2002).

The discovery and investigation of cell signaling compounds over the last decade has opened a new arena of biofilm research. First discovered in the marine bacterium *Vibrio fischeri* (Fuqua et al., 1996), cell signaling compounds are organic molecules that are produced by cells and secreted into the surrounding fluid. When present at sufficient concentrations, these signal molecules regulate gene expression in cells. Biofilm cells have been shown to respond to chemical signals (also referred to as quorum-sensing molecules) such as acyl homoserine lactones (HSLs) (McLean et al., 1997; Davies et al., 1998). These molecules induce gene regulation when a sufficient concentration of the signal compound accumulates in close proximity to the cell, a condition which is facilitated by the close packing of cells and bulk fluid flow limitations within biofilms.

Recent work assessing the genetic and proteomic conditions of attached cells has renewed the question of what constitutes a biofilm. Biofilms have often been identified as having a characteristic structure and resultant transport limitations (Lewandowski et al., 1991). However, it has also been recognized that gene regulation and protein synthesis are altered within attached cells, to the extent that biofilm cells can be as genetically dissimilar to planktonic cells of the same species as they are to cells of a completely different species (Stoodley et al., 2002). Within minutes of surface attachment of a bacterial cell (long before any transport limitations are present), cells begin to alter their genetic condition to encode the production of extracellular polymer and make other changes characteristic of the biofilm phenotype (Sauer and Camper, 2001). This research suggests that a biofilm is not just an accumulation of cells on a surface but is a fundamentally different condition of microbial growth. Clearly, our understanding of what constitutes a biofilm has changed as our methods of investigation have improved.

Steps in Biofilm Formation and Maturation

Biofilm formation is a complex process that has been, for convenience, traditionally divided into five major steps (Fig. 1); in reality, biofilm formation is a continuous process. The result of this is that any biofilm in an oil field system is dynamic, heterogeneous, and discontinuous, with different microareas having biofilms at different stages of development.

STEP 1: INITIAL ATTACHMENT

Biofilm formation on surfaces occurs rapidly following contact of a surface with nonsterile fluids. Typically, within minutes, a conditioning film of organic molecules develops, facilitating initial cell attachment to solid phases such as pipe walls, process plant vessel interiors, or porous media (Camper et al., 1994). Although certain pipe construction materials (such as some nonferrous metallics or polymer coatings containing imbedded antimicrobials) have been observed to delay the onset of cell attachment and biofilm formation in laboratory experiments, no surfaces have yet proven to be antifouling over a period of days or weeks. Cell attachment to previously uncolonized surfaces can also occur through the redistribution of attached cells via surface motility (Dalton et al., 1996). In this case, clusters of cells may migrate across a surface due to hydrodynamic pressures (Stoodley et al., 1999b) or individual cells may migrate through twitching motility (a process by which bacteria move through the extension and subsequent contraction of cell surface pili) (O'Toole and Kolter, 1998). In addition to the above mechanisms, cell clusters previously

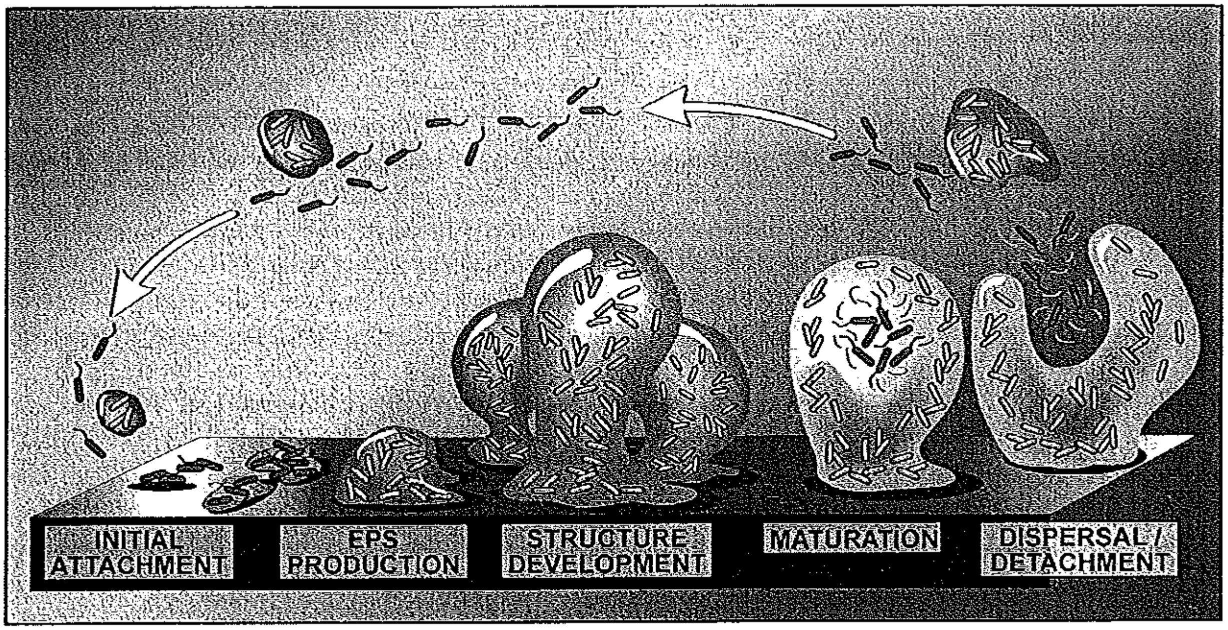

FIGURE 1 Schematic model for attachment of planktonic cells to a surface and growth of microcolonies, followed by detachment and reattachment of cell clusters.

detached from an upstream biofilm may reattach en masse, with each cluster containing hundreds or perhaps thousands of individual cells. Clearly, this phenomenon accelerates the process of biofilm initiation.

STEP 2: BIOFILM INITIATION AND EPS PRODUCTION

Following initial surface attachment, many species of bacteria undergo a shift in protein synthesis, which results in the production of extracellular polymers that serve to irreversibly anchor the cell to the surface. Physiologic and metabolic changes resulting from transition of the cell from a planktonic to a sessile state have led to the identification of a biofilm phenotype (Sauer and Camper, 2001). This phenotypic change may be accompanied by a lag phase of several hours as the cell up-regulates biofilm-specific protein synthesis (Rice et al., 2000). Major observable changes in cell phenotype as a result of cell attachment include the production of extracellular polymer and the loss of flagella, and comparison of proteomic analysis of *Pseudomonas putida* under sessile and planktonic conditions showed that attachment resulted in 15 up-regulated proteins and 30 down-regulated proteins, confirming major changes in bacterial metabolic pathways upon attachment to a surface (Sauer and Camper, 2001).

EPS production serves to build a three-dimensional matrix in which cells may occupy <15% of the total biofilm volume (Costerton and Stoodley, 2003). This three-dimensional structure influences the movement of dissolved chemical species into and out of the biofilm, entraps particulate material and cell clusters from the bulk fluid, and creates diffusion gradients that lead to localized water chemistry conditions, which may vary significantly from the bulk fluid interface to the substratum (Lewandowski and Beyenal, 2003).

STEP 3: BIOFILM STRUCTURAL DEVELOPMENT

The early simplistic model of a multilayered structure of biofilms developed in the 1980s (Wanner and Guyer, 1986) has been replaced by a more complex model for mature biofilms. Recent advances in techniques such as microscopy, microelectrodes, biochemical markers, and immunofluorescent staining have shown that as biofilm matures, bacterial growth tends to concentrate in cell clusters, a condition which leads to the development of a complex biofilm architecture, as shown in Fig. 2 (Stoodley et al., 1999c; Lewandowski, 2000). Between cell clusters, flow channels carry dissolved and particulate material from the bulk fluid to the interior of the biofilm by mass transport, at the same time carrying away waste products. By contrast, diffusive flow dominates within clusters, and thus the biofilm microstructure influences cell activity locally, with cell growth rates related to their proximity to flow channels and consequent nutrient supply (de Beer et al., 1994a). Bacteria that thrive in environments different from bulk fluid conditions may actively grow only within cell clusters away from flow channels. For example, anaerobes in a biofilm in contact with an aerobic bulk fluid tend to be active only deep inside cell clusters (where the oxygen concentration is lowest). Conversely, obligately aerobic bacteria will predominate in the outer layers of cell clusters, where oxygen concentrations are highest.

The geometry of the substratum is another factor that contributes to biofilm structural development. Small imperfections in a pipe wall, such as scale deposits or pits, may harbor populations of bacteria for which access to reducible metal species is more critical than access to organic carbon, such as iron- and manganese-oxidizing bacteria (Dickinson and Lewandowski, 1996). In porous media, environmental conditions can vary significantly over very short distances. The complex nature of reservoir rock in oil formations after water injection or interstitial pore spaces in soil or fractured rock matrices leads to a variety of physiological niches within a seemingly homogeneous system (Sturman et al., 1995).

Although biofilm structural development is clearly influenced by cell growth factors, such as the species present, growth rate, and EPS

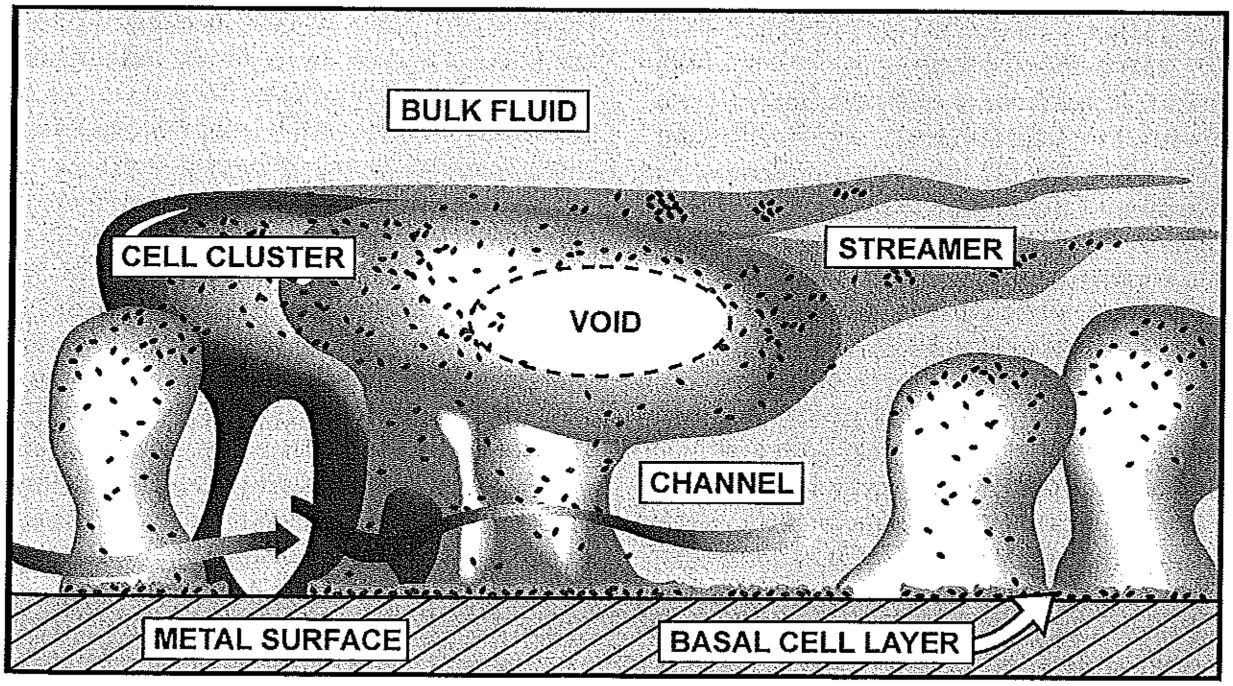

FIGURE 2 Schematic model of the complex microstructure and architecture of a mature biofilm developed on a surface. The biofilm is composed primarily of secreted polymeric substances, with bacterial cells being embedded (and thus protected) in cell clusters. The presence of channels and voids assists mass transport and diffusion of nutrients and waste products, to maintain the activity of the biofilm.

production, fluid hydrodynamics may also play an important role in determining biofilm structure. Biofilms grown under laminar flow conditions, such as in cooling towers or low-flow pipelines, generally assume the familiar tower-and-flow channel appearance described above (Fig. 2), while those grown in a high-shear environment, such as injection wells or high-velocity pipelines, tend to become elongated in the direction of flow, often forming filamentous streamers (Stoodley et al., 1999a). Additionally, biofilms grown in a high-shear environment are generally denser, more rigid, and structurally stronger than those grown under low-shear conditions (Liu and Tay, 2001; Stoodley et al., 1998). Biofilm growth conditions therefore influence possible biofouling eradication mechanisms, both from the standpoint of chemical biocide efficacy and physical means of removal. Denser biofilms are more resistant to the diffusive flow of chemical agents, as well as being more tenacious in their adherence to pipe walls.

STEPS 4 AND 5: BIOFILM MATURATION AND DETACHMENT

Biofilms are ever-changing entities, but biomass concentration typically reaches stasis after a period of days or weeks, if left untreated. Depending on the species composition and structure of the biofilm, bacteria located deep within a cell cluster may enter a stationary phase (Hengge-Aronis, 1993). This condition of reduced activity has been shown to make cells less susceptible to antimicrobial treatments that depend on active cell growth, such as antibiotics (Anderl et al., 2003), and more likely to detach from the substratum (Lamed and Bayer, 1986). Biofilm maturity is characterized by periodic detachment events, which include the release into the bulk fluid of either individual cells or multicell clusters. As a result of these detachment events, overall biofilm thickness may remain in a quasi-static state in the mature biofilm. Recent biofilm studies have shown that cell detachment can occur as what has been termed swarming dispersal, where an inner region of a cell cluster liquefies, with the interior biofilm cells reverting to the planktonic state, complete with flagella. These cells then "swim" from the biofilm into the bulk fluid, completing the biofilm life cycle (Sauer et al., 2002; Tolker-Nielsen et al., 2000). In many environmental systems, bacterial predation in biofilms may occur from higher organisms such as protozoa, increasing the complexity and heterogeneity of the biofilm on a metal surface (Murga et al., 2001).

BACTERIAL INTERACTIONS IN BIOFILMS

Biofilms represent microscale ecosystems. In many cases, these ecosystems contain a wide range of environmental niches, from aerobic to microaerophilic to anaerobic, from copiotrophic to oligotrophic, and from heterotrophic to chemolithotrophic. The ability of cells in biofilms to modify their surroundings results in local gradients of key parameters such as oxygen, pH, redox potential, nutrients, and flow rate; these bacterially generated gradients in turn provide a variety of potential growth niches for other microorganisms. In addition, the close proximity of cells in biofilms offers an ideal condition to facilitate intercellular exchange of genetic material and chemical signals, resulting in a stable, multispecies, complex, interactive consortium of bacteria.

Competition and Mutualism in Biofilms

As biofilms mature and become structurally more complex, a variety of growth niches form due to the development of concentration gradient, as described above. Within a particular microniche, interspecies competition occurs, which typically results in the dominance of the organism capable of the fastest growth. However, in laboratory experiments with defined cultures, it has been noted that such competition does not result in the eradication of the slower-growing organisms, despite their disadvantages in growth rate (Sturman et al., 1994; James et al., 1995; Stewart et al., 1997). Bacteria can survive for extended time periods under adverse conditions by forming dormant structures such as spores and ultramicrobacteria (Lappin-Scott et al., 1994). These dormant forms can become active as soon as the environmental conditions become favorable again, explaining both the ubiquity of microorganisms globally and the quick colonization of available niches (Costerton et al., 1995).

Biofilm growth therefore generates various niches (at both the micro- and macroscale levels), and the best-adapted organisms establish active populations in each. It is likely that both competitive and, to some extent, mutualistic interactions occur between species in these niches. An example of macroscale niche development is the growth of denitrifying organisms downstream from the aerobic zone in a seawater injection system. At the microscale level, inhabitants of the same biofilm exhibit mutualism when one member of the consortium consumes a compound that is toxic or inhibitory to another member (e.g., oxygen consumption by aerobic bacteria in close proximity to active sulfate-reducing bacteria [SRB]) or produces a by-product that enhances the activity of a neighboring cell. In addition, exchange of genetic material can occur by plasmid transfer between cells in close physical proximity in biofilms, conferring resistance to some antimicrobial agents. The complex nature and dynamics of biofilms in natural and constructed systems ensure that there are a large number of microniches available to support the growth of a wide diversity of microbial types. Diffusion gradients of oxygen, organic carbon, reaction products, and nutrients form as the biofilm develops (Costerton et al., 1995), providing opportunities for bacteria to grow within the biofilm, even when bulk fluid conditions are not conducive to their activity. Metabolic cooperation within biofilms has been described in complex environmental systems that generate methane (Macleod et al., 1990) and degrade cellulose (Kudo and Costerton, 1987). In industrial systems, particularly ferrous-based pipelines, biofilm-enhanced mutualism can lead to the proliferation of corrosion-causing bacteria beneath less harmful populations (Dickinson and Lewandowski, 1996).

Genetic Exchange in Biofilms

The exchange of genetic material (DNA) between bacteria is a fundamental mechanism in evolution and microbial adaptation. Genetic exchange can occur via plasmid transfer, via transposition and conjugation, or simply as a result of nucleic acids from a lysed cell being taken up by a living cell (Angles et al., 1993). Such DNA transfer occurs routinely in all cells, planktonic or sessile, but the proximity of cells within cell clusters in biofilms facilitates this process for attached bacteria. Because biofilms contain cell densities many times those observed in planktonic cells, rates of gene transfer may be very high in biofilms (Ghigo, 2001). This genetic exchange may transfer antibiotic resistance or the ability to metabolize an uncommon substrate, or the exchange may influence the growth and survival envelope of bacteria within the biofilm. Stoodley et al. (2002) have suggested that the complex biofilm structure may facilitate gene transfer between cells, a function that may in turn be regulated by signaling molecules.

Cell Signaling in Biofilms

While cell signaling is a relatively new area of microbiology research, it has received a great deal of attention in the past several years, particularly as a potential means of disrupting biofilm formation or facilitating biofilm detachment (Stoodley et al., 2002). Microbially produced quorum-sensing molecules, such as HSL, induce gene regulation when a sufficient concentration of the signal compound accumulates in close proximity to the cell (Fig. 3). Quorum sensing has been linked not only to biofilm formation (Davies et al., 1998), but also to the expression of virulence factors in a variety of opportunistic pathogens (Hentzer and Givskov, 2003). The identification of HSLs as important signaling molecules has led to the investigation of HSL analogs, which could potentially function to confuse the receiving cell by blocking signal receptors. Not surprisingly, the search for signal analogs has focused mainly on human pathogens, such as *Pseudomonas aeruginosa* in cystic fibrosis, where

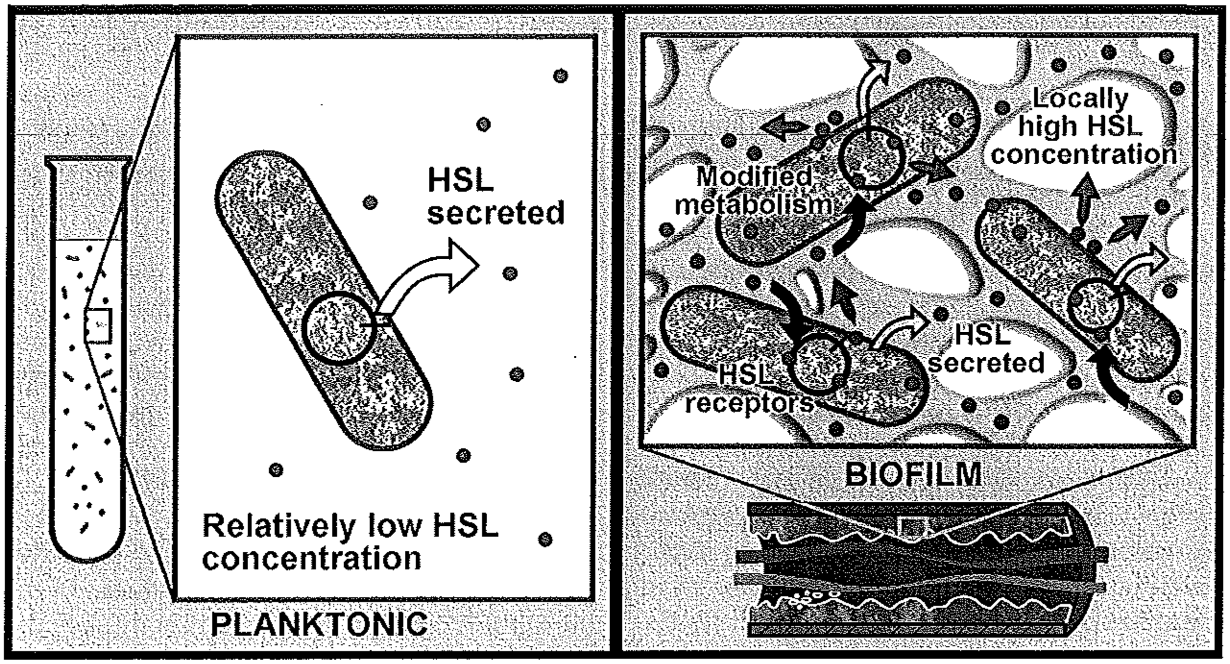

FIGURE 3 Cell-cell communication (quorum sensing) in bacteria is associated with the accumulation of signal molecules such as HSLs that coregulate gene transcription. Communication may be inter- or intraspecies, and a wide variety of cellular functions (such as metabolic pathways, growth rate, and detachment) may be influenced in other bacteria by the secretion of signal molecules. The biofilm matrix enhances cell-cell communication, since bacterial cells are in close proximity to each other.

development of an effective drug therapy could have immediate and lifesaving implications.

Potential applications of quorum-sensing bacterial inhibition to industrial settings will be more challenging than infection control in medical contexts for several reasons, including the expense of potential signal molecules and the complexity of real-world industrial biofilms. Although response to a common signal (cross-talk) has been established on a limited basis between different bacterial species (Riedel et al., 2001), the challenge of biofilm modification in an industrial setting, with a variety of microbial species, corrosion products, inorganic molecules, and variable chemical conditions, has yet to be approached in laboratory investigations, let alone field applications.

Biofilms clearly are exquisitely complex communities that adapt readily to changing conditions, cling tenaciously to virtually all solids, and possess defense mechanisms (both individually and en masse) that allow them to persist in the presence of antimicrobials that effectively kill their planktonic counterparts. Signaling molecules are part of this collective behavior, but it is as yet unknown to what extent the many bacterial species in an ecosystem (such as an oil-bearing formation or a petroleum pipeline) respond to common signals or influence the behavior of neighboring cells. The investigation of signal analogs holds promise for future biofilm control efforts but is in its infancy with regard to the complex and highly variable conditions presented in the petroleum industry.

MONITORING BIOFOULING IN THE PETROLEUM INDUSTRY

Laboratory Biofilm Studies

Industry and academic researchers have long recognized that the essential problem of studying petroleum industry biofilms in the laboratory is one of finding the correct balance between simplicity (and thereby controllability) and relevance. Implicit in finding this balance are questions of system scalability, microbial consortium complexity, and system variability (dynamics). To address research questions ranging from investigations of microscale events to overall system behavior, researchers have developed a battery of growth reactors and methods. Microscale studies to determine cell attachment rates, biofilm behavior, and biocide penetration are typically performed in simple flow chambers that can be placed on a microscope stage and viewed throughout the experiment (Stoodley et al., 1999b). While flow cells offer a high degree of control over fluid hydrodynamics and valuable microscale data collection options, the biofilm cannot be physically sampled, and only simple systems can be approximated. A step up in complexity is the continuous stirred-tank reactor (CSTR) for biofilm study. CSTRs take many forms, such as the commercially available annular and disk reactors. CSTRs are useful for studying biofilms where both planktonic and sessile growth phases are present. A standard biofilm method has been developed using such a reactor system and standard biofilm enumeration protocols (American Society for Testing and Materials, 2001). CSTRs typically have several easily removable coupon surfaces from which a biofilm is enumerated or viewed via microscopy. More complex yet, and commensurately closer to field conditions, are pipe loop reactors and medium-filled column reactors. Pipe loop systems can often be operated under conditions quite similar to those found in field situations. Biofilm sampling is more difficult in these systems than in the CSTR reactor, but it can be accomplished through installation of threaded, flush-mounted pipe wall insertions that can be removed individually. Columns filled with sand, soil, or other media represent the extreme end of the spectrum in terms of complexity and field relevance. They are, however, difficult to control and sample. Bulk fluid properties can be easily sampled in the influent and effluent, but biofilm sampling necessarily involves removing a portion of the solid phase and recovering the attached biomass. Overall, the correct reactor system for experimental work must be chosen based on the need for a field relevant system and the need to control data quality.

Assessment of Biofouling in the Field

Collection of representative biofilm samples and valid measurements of biofouling rates are major challenges for oil field systems. The problems of collecting and reliably analyzing water samples are well known (American Petroleum Institute, 1982; McInerney and Sublette, 1997), and such samples have only marginal relevance to biofouling assessments (Sanders, 1992). Industry standard practices focus on methods for determination of planktonic bacterial numbers and only briefly mention sampling and testing for the more important biofilm components of a system (National Association of Corrosion Engineers, 1990, 1994). In-line and real-time monitoring of bacterial attachment, biofilm accumulation rate, onset of microbial corrosion, or some other associated parameter in the most critical part of the system is the ultimate goal of biofouling management. Ideally, this should be linked automatically to a control measure such as bactericide injection. While a wide range of potential technologies are available or are being developed for rapid quantification of biofouling (Nivens et al., 1995; Flemming, 2003), very few of these are at the stage of field deployment; they are suitable for laboratory use until they can be made simple to use, install, interpret, and maintain (Borenstein and Licina, 1994).

CURRENT BIOFILM ASSESSMENT METHODS

If biofilms are assessed at all in oil field systems, traditional cultivation techniques are usually applied to samples from sidestream biofouling devices connected to the process stream, from corrosion coupons or special in-line biocoupons. Sidestream and in-line biofouling monitors are available from numerous vendors and are essential tools in any biofouling control program. While it is possible to collect frequent samples from sidestream systems, care must be taken to ensure that the linear flow rate in the sidestream matches that of the line, so that a representative assessment can be made. In-line biocoupons have the advantage that the biofilm is more likely to be representative of the system, but access fittings are not always available at the most useful location, and sample frequency may be limited. Another possibility suggested by Jenneman et al. (2004) is to use porous media "biotraps" in sidestreams. Using porous polymer beads gives a large surface area for bacterial attachment and speeds up biofilm development so that molecular and cultural methods can be conducted to assess the degree of biofouling in the system.

Having collected a biofilm sample on a surface, the challenge of undertaking a representative analysis begins. Current cultivation methods are limited by (i) the difficulty of preparing a suspension of single cells from the tightly bound cell clusters in the biofilm while retaining viability, (ii) the selectivity of cultivation media, (iii) the time taken to obtain results, and (iv) the semiquantitative nature of the methods (Maxwell et al., 2004). Biofouling assessment methods based upon changes in heat transfer resistance, differential pressure, or optical attenuation have been developed for other industries, usually for sidestream systems, but these have not yet been applied to oil industry systems due to their complexity and cost (Ludensky, 1999).

DEVELOPING BIOFILM ASSESSMENT METHODS

Biofilm monitoring techniques can be classified into three groups, depending on the level of information they give (Flemming, 2003).

- Level 1 monitors detect the buildup of surface fouling in general and cannot differentiate between biomass and inorganic fractions. Methods such as optical sensors, ultrasound, heat transfer resistance, and quartz microbalances fall into this category.
- Level 2 monitors differentiate organisms and/or biomass from inorganic deposits. Biochemical probes, genetic methods, and confocal laser microscopy fall into this category.
- Level 3 monitors detect the activity and viability of bacteria. Few monitors currently fall into this category, but some of the genetic, chemical, and enzymatic methods might be developed for this.

There are many developing technologies with the potential for obtaining real-time indications of bacterial numbers and colonization rates on surfaces. These have primarily been developed for clean water situations, such as the nuclear power industry, where any biofouling results in poor performance of the plant, but some have potential for specialized studies in oil field systems. Scanning confocal laser microscopy and fluorescence in situ hybridization, combined with denaturing gradient gel electrophoresis, are now in routine use to monitor biofilms in heating systems and could be directly applied to the oil industry. (Kjellerup et al., 2003).

An automated biofouling control system has been used in recirculating systems, with a fluorogenic bioreporter chemical added. This complex changes its fluorescence emission spectrum when it interacts with biofilm or bacteria and continuous analysis of in-line samples is possible. Changes in fluorescence peaks are related to the degree of microbial activity, and the bactericide pump rate can be linked to the bioreporter signal so that real-time, automated, biofouling control can be imposed. (Chattoraj et al. 2002). This has been demonstrated as a practicable tool to link real-time, on-line monitoring with the bactericide pump so that there is a direct feedback between the monitor and biofilm control measures. Electrochemical methods are developing rapidly, such as the BIOX probe for cooling water systems (Mollica and Christiani,

2003) and the Biofilm Activity Monitoring system (Veazey, 2003). The BIoGEORGE system encourages the attachment of bacteria to a complex electrochemical probe, measuring the decrease in polarization resistance due to biofilm accumulation and detecting the current flow between identical nonactive electrodes established by sessile bacterial activity. This can give advanced warning of the onset of biofouling; by linking the probe to a bactericide dosing pump, the system is treated only when the risk of biofouling is high. Methods under development are listed in Table 1. The ultimate goal is to have a usable, robust system that will monitor biofilm buildup in line and in real time; there are already a few systems which show promise in field applications. Our ability to detect and control biofouling in oil field systems will undoubtedly improve, providing that the technology is simple, robust, and cost effective.

PRACTICAL IMPLICATIONS OF BIOFILMS IN THE OIL INDUSTRY

With the exception of their uses in wastewater treatment systems, MEOR, and bioremediation, biofilms typically result in problems requiring costly and ongoing remedial measures. Furthermore, it is well documented that cells growing within a biofilm are more highly resistant to antimicrobial treatments than planktonic cells. When exposed to a particular antimicrobial dose, the log reduction in planktonic cells is typically 2 to 4 orders of magnitude higher than is the case for biofilm cells. In extreme cases, this difference may be over 8 orders of magnitude (Stewart et al., 2000; Sanders, 1988). Research over the past 20 years suggests three reasons for this heightened resistance: (i) depletion of the antimicrobial in the bulk fluid adjacent to a biofilm, (ii) failure of the antimicrobial to adequately penetrate the biofilm, and (iii) reduced

TABLE 1 Methods being developed for biofilm assessment in oil field applications[a]

Methods suitable for use in industrial systems	Methods currently limited to laboratory studies
Fiber optic devices built into pipewalls (use NAD/NADP autofluorescence)	X-ray PES for EPS detection
Differential turbidity	Microelectrodes (pH, nutrients)
Rototorque annular reactor (off-line)	Conductimetry
Heat transfer resistance	Scanning confocal laser microscopy
On-line ultrasound probe (ultrasonic frequency domain reflectometry)	Differential interference contrast microscopy
ATP	Photoacoustic spectroscopy
Lasers (focused-beam reflectance)	Microautoradiography
Infrared on-line detectors	Beta microimaging
Quartz crystal microbalance	Phospholipid fatty acid markers
Fluorescence in situ hybridization, in situ PCR	EPS chemistry (lectin binding)
Denatured genome gel electrophoresis	Enzyme activity (fluorescent reporter)
Viable stains (DAPI, CTC, EMA, etc.)	Titrimetry (pH stat)
Electrochemistry (ECN, BIOX probe, BIoGEORGE probe)	Terminal restriction fragment length polymorphism
Redox potential probe	Natural fluorescence (NADP-tryptophan)
Cyclic voltametry	Green fluorescent protein tags
Chemical changes (for effluent treatment plant)	Fluorescence spectrometry
BioWatch (Ondeo) used to control process, based on optical density of film	
BioSensor (Ondeo), a bioreporter chemical addition for a recirculating system	

[a] DAPI, 4',6'-diamidino-2-phenylindole; CTC, cyanotolyl tetrazolium chloride; EMA, ethidium monoazide; ECN, electrochemical noise; PES, photoemission spectroscopy.

susceptibility of biofilm bacteria to antimicrobial treatment. In the first case, the higher cell density of the biofilm relative to cell suspensions renders a reactive biocide less effective against the biofilm. In the second case, an antimicrobial reacts with a component of the biofilm (or a cell excretion) and is neutralized before diffusion carries it to the substratum. This mechanism has been shown to occur with oxidizing agents such as hypochlorite and hydrogen peroxide (de Beer et al., 1994b; Xu et al., 1996; Sanders and Robinson, 1992) as well as for enzymatically degraded antibiotics (Nichols, 1991). In the third case, the antimicrobial may sufficiently penetrate the biofilm, but it is nonetheless ineffective, due to biofilm cells existing in a slow-growing or starved state (Brown et al., 1988) or as an antimicrobial-resistant phenotype (Gilbert and Brown, 1995). While biofilm control is possible with a variety of oxidizing and nonoxidizing biocides and physical removal techniques, the protective mechanisms outlined above hamper efforts to completely eradicate biofilms in virtually all industrial and environmental systems. Repeated antimicrobial dosing or physical cleaning is therefore necessary, as biofilm regrowth following treatment typically occurs over a period of hours to days.

Because biofilms are in intimate contact with pipe walls, vessel interiors, and environmental solids, their capacity to modify these surfaces is greatly enhanced relative to that of planktonic cells. The acceleration of corrosion due to sessile bacteria (microbially influenced corrosion) has been of paramount interest to industrial practitioners and researchers alike and is described in detail in chapter 8. Biofilms facilitate corrosion both by trapping corrosive metabolic products in close proximity to surfaces, as in the case of hydrogen sulfide produced by SRB (Geesey et al., 2000), and through the metabolism of bacteria which utilize reduced metals as a source of energy, as in the case of iron- and manganese-oxidizing bacteria (Lewandowski et al., 2002). Recent work by Lewandowski et al. (2003) suggests that the activity of manganese-oxidizing bacteria inside biofilms can greatly enhance corrosion through the cycling of relatively low concentrations of dissolved manganese. In this mechanism, dissolved Mn^{2+} is oxidized to $MnO_2(s)$ by manganese-oxidizing bacteria in biofilms. MnO_2 surface deposits then oxidize passive metallic pipe materials, cycling manganese back to Mn^{2+} to begin the cycle anew.

Some workers have postulated that protective biofilms may prevent corrosion under some circumstances. Pure cultures of several aerobic and facultative bacteria (grown under aerobic conditions) were shown to decrease corrosion rates relative to sterile controls in laboratory studies (Jayaraman et al., 1997). Preliminary field studies using a similar strategy of inoculation of a beneficial bacterium (*Bacillus polymyxa*) have shown a positive effect on corrosion rates (Syrett et al., 2002). A key issue in such a treatment strategy is the survival of the inoculated organism(s) into a nonsterile environment. Field application of this strategy would likely require either continuous or repeated inoculation of the beneficial consortium to maintain its dominance over indigenous bacteria. Nonetheless, this strategy may hold future promise as an acceptable alternative to the conventional bactericide treatments to control biofilm-related problems in oil field systems.

Biofilms may grow to the extent that accumulated cells and extracellular polymer influence the hydraulic or thermal conductivity of their environment. This phenomenon is particularly problematic in porous media, injection wells, membrane systems, and heat exchangers. The propensity of biofilms to reduce hydraulic conductivity has been used in the construction of biological barriers to assist groundwater bioremediation. Nutrient injection has been shown to decrease hydraulic conductivity 100-fold in field studies and over 10,000-fold in laboratory reactors (Cunningham et al., 2003). The detrimental effects of biofilms in oil field systems are covered in greater detail below.

Microbial cell activity changes the water chemistry of the media in which the cells grow. These changes may be particularly acute in the case of biofilms because cell densities are far greater than with planktonic growth, especially when the surface area/fluid volume ratio is high or when linear flow rates are low. In addition to removing organic carbon, biofilms may add detrimental chemical compounds to the solution, such as hydrogen sulfide in the case of SRB activity, or alter the physicochemical properties of the fluid, for example, by pH modification or oxygen removal. Although the activity of planktonic populations is well understood in this regard, the effects of biofilm activity in industrial systems are less well studied. Since biofilms may harbor unknown organisms or support difficult-to-kill populations, they are of particular importance with respect to understanding the impact of microbial growth in specific oil field systems.

The most important consequences of uncontrolled biofilm growth in an oil field system are negative: they directly impact the safe operation, economic performance, and environmental compliance in all areas of extraction, transport, refining, and distribution (Sanders and Hamilton, 1985). Sometimes, microbial activity in an industrial system can be beneficial, if it can be predicted and controlled. Numerous attempts have been made to utilize microbes to improve oil recovery, reduce production costs, or minimize environmental impact. The widespread impact of biofilms on the oil industry is summarized in Fig. 4, and these effects are listed below.

1. Water injection system fouling and/or microbial corrosion. Water (seawater, aquifer, or produced water) is treated before injection into the oil formation for secondary oil recovery. Bacteria grow in the water and on the pipe walls, resulting in downstream contamination and microbial corrosion of carbon steel and other materials.

2. Downhole microbial corrosion (mesophiles). When bacteria are present in the injection well, they form biofilms, which result in microbial corrosion of the tubing, casing, and subsurface safety valves. Injection water is typically cool, so mesophilic bacteria are involved, growing at less that 45°C.

3. Reservoir souring and plugging. If bacteria survive the transition from injection water to the formation, they attach in the near-well-bore region. Subsequently, they may migrate deeper into the formation, and thermophiles (growing only at temperatures in excess of 45°C) may then grow. In both locations, SRB will form H_2S, and a biofilm will plug the rock, leading to a loss of injectivity and poor recovery of oil.

4. Downhole microbial corrosion (thermophiles). Downhole production well materials can be colonized by thermophilic bacteria derived from the formation. This results in microbial corrosion attack of the tubing and/or casing; (most critically) the corrosion-resistant alloys of subsurface safety valves and wellhead wing valves, with obvious safety implications; and electrosubmersible pumps.

5. Production system microbial corrosion, H_2S, and oil-in-water problems. Produced fluids and gas are separated in process trains and gas-oil separating plants. Bacteria colonize the water-wet surfaces of these facilities, and this can result in microbial corrosion. In addition, H_2S can be generated in significant quantities in production systems, adding to the H_2S produced downhole. Furthermore, bacteria and iron sulfide stabilize oil-in-water emulsions, leading to poor separation and a high oil-in-water content in discharged or reinjected produced water.

6. Subsea manifold microbial corrosion. Increasing use of subsea completions, templates, and manifolds results in reduced opportunity for chemical treatment, repair, or replacement. Even a small amount of fouling or microbial corrosion in these systems has a major economic impact.

7. Flow line internal microbial corrosion and fouling. Subsea water injection and production flow lines commonly suffer from microbial corrosion and biofouling due to the low linear flow rates and deposit accumulation. The same is also true for onshore flow lines, which may be in excess of 100 km long, with

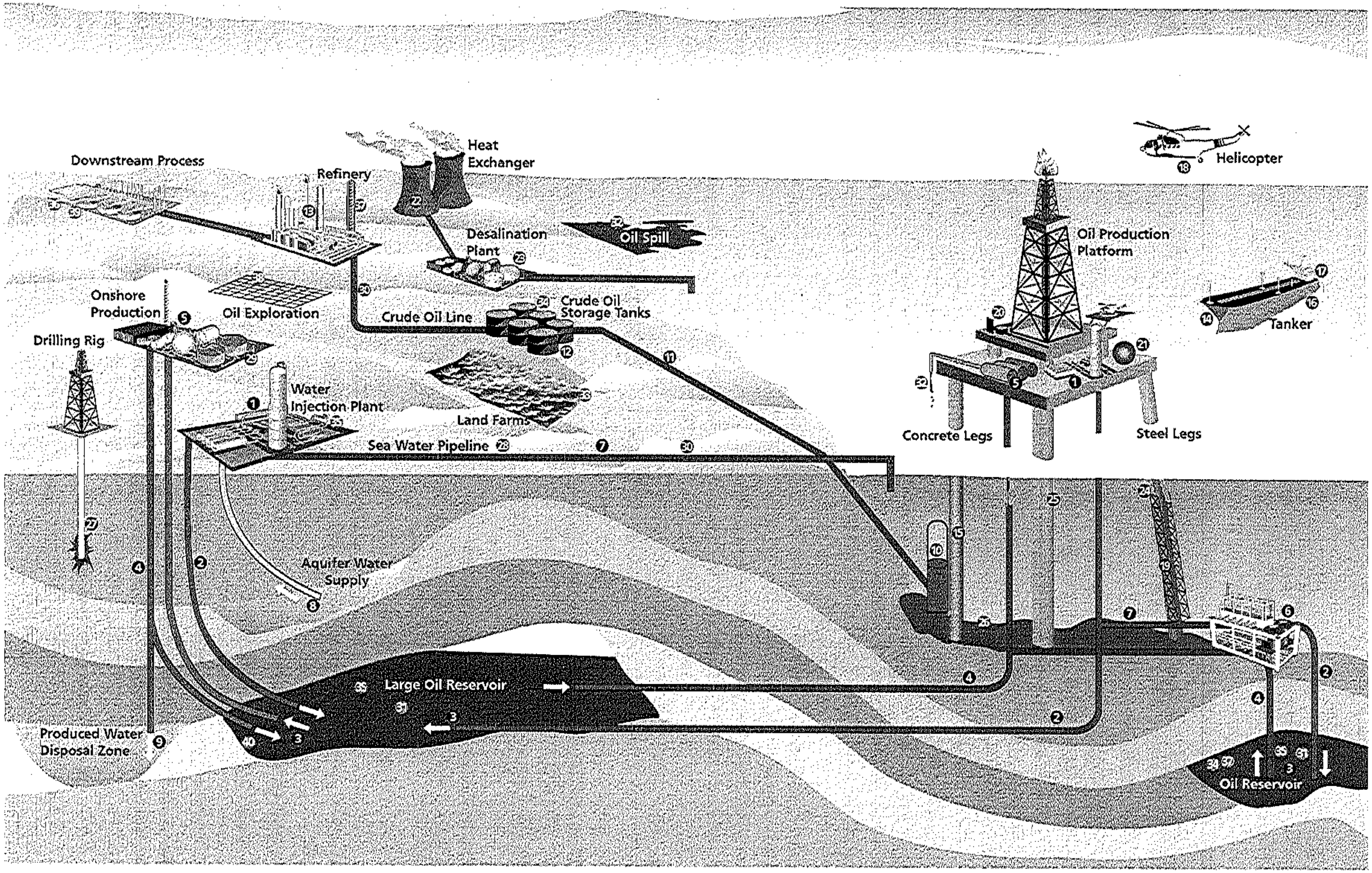

FIGURE 4 Positive and negative consequences of biofilm growth in the oil industry. For details on each numbered biofilm effect, see the text.

complex distribution systems. Problems tend to materialize toward the far end of these systems and are usually at the 6 o'clock position.

8. Aquifer supply plugging and electrosubmersible pump microbial corrosion. Aquifer water may be used as a source for water injection. Microbial growth in the borehole or near-well-bore area can result in plugging of the rock and therefore reduced water production. It can also result in microbial corrosion of the electrosubmersible pump impellers and column pipe.

9. Produced water injection well plugging. Produced water may be reinjected after treatment for pressure maintenance or into another formation for disposal. Bacteria in the produced water will contaminate the injected zone, leading to plugging (loss of injectivity), H_2S souring, and microbial corrosion problems.

10. Crude oil storage H_2S and H_2SO_4. Bacterial growth in offshore crude oil storage tanks initially results in organic acid production and acid attack of materials. As conditions change, H_2S is produced by SRB. In special cases, sulfuric acid is produced by sulfide-oxidizing bacteria, leading to safety and corrosion concerns.

11. Pipeline internal microbial corrosion and fouling. Even though crude oil is dehydrated, water will drop out in long distribution lines. Bacterial growth in this stagnant water results in pitting in the 6 o'clock position, ultimately creating full penetration and a groove and requiring replacement of the line.

12. Onshore crude oil tank microbial corrosion. Water inevitably collects in the bottom of crude oil storage tanks, and bacteria (and fungi) grow quickly on the soluble components of the oil. Dense microbial populations result, with possibilities for microbial corrosion of poorly protected tank surfaces and contamination of downstream systems.

13. Refinery microbial corrosion. Refineries handle a wide range of hydrocarbons. Although refinery processes are at high temperatures in the absence of water, storage of the refined products allows the possibilities for microbes to grow if water condensation occurs. Gas oils, paraffins, and the low-boiling-point fractions are most susceptible.

14. Crude oil cargo tank microbial corrosion. Marine tankers invariably have a buildup of water in the cargo tank bottoms, which is difficult to drain. This water may be from condensation or from the small amount of water introduced with the oil. Bacteria and fungi grow in this water and pose a risk of microbial corrosion to unprotected metal surfaces.

15. Diesel tank contamination and spoilage. Diesel and gas oils are readily degraded by bacteria and fungi. When stored for extended periods, the microbes can become suspended in the hydrocarbon and eventually lower the combustibility of the fuel. Storage tanks for standby generators and lifeboats are particular problems due to low fuel consumption under normal operations.

16. Ship fuel fouling, spoilage, and microbial corrosion. Fuels used for marine engines can easily become contaminated with microbes, due to the diverse bunkering locations. Seawater displacement systems are sometimes used to balance the trim of the vessel: these suffer fuel injector corrosion and blockage, high fuel filter consumption, and fuel system microbial corrosion.

17. Ship lubricating and hydraulic oil contamination. Lubricating and hydraulic oils can become contaminated by bacteria, particularly when water is present as a contaminant. Bacterial activity reduces the performance of the oil, shortens its lifetime, and causes engine damage or failure of hydraulic systems.

18. Helicopter and aircraft fuel contamination. Aviation fuels are readily used by hydrocarbon-degrading microbes in the presence of water. Microbial growth can quickly make the fuel unusable, due to increased solid matter (particularly fungal hyphae), reduced pH, and lower combustibility.

19. Water-filled steel legs and hydrotest microbial corrosion. Any system filled with untreated water (even potable water) can suffer from biofouling. This applies to ballast water tanks, potable water tanks, and hydrotest fluids used for pressure testing. Bacteria are more likely to form a biofilm in nutrient-poor water systems, thus initiating microbial corrosion.

20. Firewater system microbial corrosion and fouling. Firewater systems tend to remain full of static water for long periods. This encourages the growth of biofilms; particularly in seawater-filled systems, microbial corrosion, sulfidation, and biofouling can occur. In extreme cases, microbial growth may be severe enough to block the sprinkler heads.

21. Potable water microbial corrosion and pathogens. Potable water and water-filled heating systems can harbor a significant bacterial biofilm. This biofilm can cause intense pitting corrosion of copper piping and the replacement of infected systems. The biofilm also harbors pathogenic microbes such as *Legionella,* posing a health risk.

22. Heat exchanger microbial corrosion and fouling. Heat exchangers are particularly prone to bacterial fouling and microbial corrosion, due to the recirculation of fluids, temperature cycling, and atmospheric exposure. Microbial corrosion and severe fouling of even corrosion-resistant alloys such as stainless steels in shell-tube heat exchangers are common.

23. Desalinization plant and reverse osmosis plant fouling and microbial corrosion. Desalinization of seawater to produce potable water requires the pumping of water through reverse osmosis membrane bundles. These can easily become colonized by microbial films, significantly reducing the efficiency and lifetime of the membranes. There is also the increased risk of microbial corrosion, due to biofilm formation on supply pipe work.

24. Microbial corrosion of steel under marine growth. Marine growth on harbor piling and offshore structures provides a protected, organic-rich environment where microbes can thrive. Large numbers of bacteria develop in biofilms on the metal surface, leading to enhanced corrosion and extra demand on cathodic protection systems.

25. Spalling of concrete under marine growth. Bacterial biofilms develop underneath marine growth on concrete structures. Acid production by microbial activity at the concrete surface can dissolve cementation and cause flakes of concrete to be detached. In severe cases, this can expose the reinforcing bars and further reduce the structural integrity of the concrete.

26. Discarded drill muds, microbial corrosion, and environmental contamination. Drilling muds contain oil, polymers, and other additives, which support bacterial growth. When discarded on the seabed, the microbial flora can be spread for large distances around the platform. Nearby seabed structures can be covered by such muds, and these are then exposed to increased microbial corrosion risk.

27. Drilling and workover fluid contamination. Components of the drilling muds, workover brines, and stimulation fluids can be readily contaminated by bacteria. In addition to causing microbial corrosion of the metallic components, loss of such contaminated fluids into the hole can contaminate the drilled formation and cause problems for the production or injection systems.

28. Naturally occurring radioactive material concentration by SRB. Uranium reduction from soluble to insoluble states can take place through the activity of metal-reducing bacteria and SRB. Although uranium is present in minute concentrations in water, a surface biofilm may contain enough radioactive isotopes to be classified as a naturally occurring radioactive material, requiring special handling and safety measures during plant maintenance.

29. Production chemical spoilage. Many production chemicals contain nutrients that support bacterial growth. Nitrogen, phosphorus, and readily degradable organic compounds can be present in scale and corrosion inhibitors and demulsifiers, for example. These can be degraded during storage or distribution, resulting in poor performance and system contamination.

30. Internal and external coating degradation. Industrial coatings applied to oil field process plants may be biodegraded, due to the activity of microbes in biofilms. Acids, surfactants, and biopolymers produced by an active biofilm may degrade even the most inert coatings.

31. MEOR. Various processes have been developed that use microbes to improve oil recovery rates or efficiency. Microbial products (polymers and surfactants) can be added to the injection water; bacteria can be injected as dormant forms and encouraged to produce biofilm to divert water flow to unswept areas; and nutrients can be injected to stimulate the resident bacterial population to produce carbon dioxide (to repressure the reservoir), acid (to increase fluid movement), or surfactants (to release bypassed oil).

32. Oil spill biodegradation. Specially selected, fast-growing, hydrocarbon-degrading bacteria are available commercially. These can be applied to oil spills in the ocean or on beaches, often with an inorganic nutrient source to stimulate their activity. This technology has the potential to be more environmentally acceptable than chemical dispersants.

33. Bioremediation. Hydrocarbon-degrading bacteria can also be used to convert hydrocarbon wastes to useful organic soils. By mixing inorganic nutrients, hydrocarbon, sand, and water with a bacterial inoculum (which is typically naturally present), the hydrocarbon is degraded and detoxified.

34. Biodesulfurization. Many crude oils contain large amounts of sulfur compounds that must be removed prior to refining. Technologies are being developed which use specially selected bacterial strains to convert the sulfur and sulfide to sulfate and other more easily treated forms.

35. Competitive microbes to control microbial corrosion and souring. Recent developments have identified the possibilities of encouraging competitive microbes to grow to suppress the activities of troublesome bacteria. Nitrate-utilizing bacteria can be stimulated by the addition of nitrate to the system, and their growth inhibits SRB activity. Nitrate treatments show promise for the effective control of H_2S souring and microbial corrosion, but they do not reduce the biofouling potential of the system.

36. Biosensors. Biosensors incorporate a biological component into a sensor to monitor specific components of the process plant in real time. The biosensor may use immobilized bacteria to detect target molecules or attachment of bacteria to the surface of the sensor, indicating the biofouling potential of the system.

37. Biorefining and bioupgrading. Processes are being developed to use bacteria to convert heavy tar sand formations (presently not recoverable) to lighter oils or to upgrade the oil by converting long-chain alkanes to smaller molecules, thus improving its viscosity. Bacteria immobilized in biofilms will undoubtedly play a part in this technology.

38. Microbial prospecting. Trace seepages of hydrocarbon gas from subsurface reservoirs encourage the growth of hydrocarbon-degrading microbes in the surface soil. Soil sampling programs can identify where these populations occur and locate target areas for exploratory drilling.

39. Bacterial production of novel oil field chemicals. Biopolymers such as xanthan gums, biosurfactants, and enzymes are available commercially for oil field applications.

40. Control by specific pathogens. The introduction of specific pathogens to target troublesome biofilm bacteria has been suggested. Bacteriophages specific for SRB could be developed, with one advantage being that sufficient bacteriophages would remain in the biofilm to provide long-term control.

CONVENTIONAL BIOFOULING CONTROL MEASURES

Four general strategies are available with respect to biofouling-related problems in oil fields. In theory, biofilm formation may be prevented by creating a barrier so that microbes do not enter and colonize the system; ultrafiltration, UV sterilization, or chlorination programs are typical (in practice, it is difficult to exclude all microorganisms from a system). The rate of biofilm development may be controlled by using chemical treatments designed to inhibit bacterial attachment or growth; by altering process conditions such as flow, temperature or salinity; or by a regular, frequent, physical removal program (pigging or scraping). The effects of biofouling may be limited by employing engineering solutions, such as using coated pipe (which does not prevent biofouling but does control microbial corrosion) or designing changes to ensure high flow rates and minimal biofouling. Finally, no control of biofouling may be attempted without the associated cost consequences being accepted.

An analysis of the biofouling potential of the system, coupled with an assessment of the associated operating and capital costs, is required to select the most appropriate biofouling control strategy. This is rarely carried out in practice; the first (and often only) control measure is usually the application of a chemical microbicide to the system to limit the development of biofilm so that it does not cause an operational problem. A wide range of chemical treatment regimes have been developed to ensure that microbial problems are minimized; these rely mostly on dosing with toxic chemicals to kill and remove biofilms. Many factors affect the performance of the chemical (for example, system water chemistry, flow conditions, the thickness and nature of existing biofilms, regrowth rates, and concentration-contact time combinations), and such factors must be considered when designing an effective biofouling control program (Characklis, 1990).

A relatively wide range of bactericidal molecules exist. Chlorine or hypochlorite is frequently used as a disinfectant as it is relatively inexpensive, can often be generated on site, and can be dosed continually at low concentrations. Commercially available nonoxidizing bactericides are generally blends of a few selected active ingredients (frequently glutaraldehyde and/or formaldehyde mixed with a surfactant or tetrakis hydroxymethyl phosphonium sulfate, again blended with surfactants). Other active agents (e.g., biguanides and isothiazolones) may be more suitable for specific systems where hydrocarbons or unusual water sources are treated.

Experience has demonstrated that the traditional chemical treatment approach often has limited success in large industrial systems, even if high bactericide concentrations or extended dosage periods are employed. There are numerous reasons why conventional bactericide applications frequently fail to eliminate microbial problems, and these need to be addressed when setting up any microbial control program (Sanders, 2002).

Underdosing

Underdosing of bactericide (or an inappropriate treatment regime) is probably the most common cause of poor microbial control. Treatment regimes suitable for a clean system may fail completely if biofilms or if substantial amounts of inorganic deposits are present. Bacteria suspended in water are relatively easily and rapidly killed by low concentrations of traditional bactericides such as chlorine and glutaraldehyde. However, when bacteria grow in a biofilm, large amounts of organic polymers protect the cells from the chemical, and much higher concentrations are required to kill these sessile microbes. Many commercial bactericide blends have surfactants added to the product in an attempt to break up biofilms and enable the bactericide molecule to penetrate throughout the biofilm. Experience has shown that it often takes more than 10 times more chemical to kill sessile microbes than the same cells in the planktonic state. Some active agents react with the organic materials in biofilms and are thus unable to control sessile bacteria. Chlorine and hypochlorite, for instance, are able to kill planktonic cells rapidly in low concentrations, but they have low levels of activity in cells deep in biofilms.

Inappropriate Dose Regimens

Selection of an appropriate dose regimen for a system relies on knowledge of the kinetics of the chemical-bacteria reaction. This is not necessary for the control of planktonic cells, where there tends to be a linear relationship between concentration and contact time: a higher concentration will be effective in a short contact time, while a lower concentration will require a longer contact time. However, the relationship between contact time and concentration is more complex when treating biofilms. To treat biofilms effectively, the chemical must diffuse throughout the film to contact the bacteria (including SRB) close to the metal surface. Therefore, if insufficient contact time is allowed, even an extremely high bactericide concentration may not be effective. Conversely, if the chemical is inactivated by the organic material of the biofilm, a low concentration may not be effective, even if dosed continuously. For bactericides to be effective against biofilms, both the contact time and the dose rate must be optimized. Each bactericide will have its own window of effectiveness, depending upon its degree of surface activity, penetrating power, and inactivation by organics. For some chemicals, 1,000 ppm for 60 min may work well, whereas 100 ppm for 600 min may not; for others, the opposite may be true.

For nonoxidizing bactericides, batch treatment is normal. Such treatments reduce the bacterial contamination of the system but rarely eliminate it completely. Surviving bacteria are able to grow in the absence of bactericide, requiring the application of another batch treatment (Larsen et al., 2000). This leads to a sawtooth graph of bacterial activity with time. The interval between batch treatments is usually fixed for convenience (e.g., once per week), but this is not necessarily the optimum. Some systems require treatment more often; in extreme cases, daily batch treatments must be employed to keep microbial problems in check.

Tolerance and Resistance

Oil field water systems typically contain a very mixed and diverse microbial flora. Each species may be represented by a wide range of strains, some of which will be more tolerant to specific bactericides, due to minor variations in cell membrane, protein structure, or metabolism. Bactericide treatment is rarely 100% efficient, and the few bacteria that survive will tend to be the more resistant strains. With repeated treatment of the same chemical, a severe selection pressure will be exerted, thus encouraging the buildup of resistant strains. If one product is used to treat the system for many years, the bacterial population will develop resistance to it, in the same way that antibiotic resistance builds up in medically important bacteria. It is for this reason that bactericides should be alternated in some way. Weekly alternation of two products with different active chemistries can help to reduce the development of resistant strains. Furthermore, synergistic effects can be obtained by using a more surface-active product to loosen the biofilm, thereby enhancing the bactericidal properties of the other product. Alternation can also be carried out over a longer timescale (monthly or yearly), and it is good practice to change product active chemistries every year, rotating through a list of tested and approved products.

Bactericide Demand

As bactericide passes through the system, it will react with bacteria, organic matter, other solids, and pipe wall materials. The concentration of the active agent will therefore decrease with increasing distance through the system. Different active molecules show this reduction to different degrees, but system demand for bactericides must be considered to ensure that an effective dose remains throughout the plant. Al-Wehaimid et al. (1994) showed that, for a large water system, the system demand can be as high as 50 to 75%, depending upon the active molecule and system conditions. System demand characteristics must be considered to achieve an adequate target dose at the furthest point in the system. To achieve this target dose, additional treatments may be necessary at intervals throughout the system. Alternatively, a substantially increased dose would be needed at the upstream injection point, resulting in overtreating part of the system.

System Conditions

Each system must be individually assessed for its bactericide requirements. Past treatment regimes will also affect the resistance of the system. Even outwardly similar process plants will have a different microbial population; furthermore, small changes in the process conditions can have a dramatic effect on which bactericides will be the most effective. Therefore, a treatment strategy developed for one system cannot be directly transferred to another.

Some parts of a system may be more difficult to treat with chemicals due to the process flow characteristics. Examples are dead legs, low-flow regions, and intermittent water drop-out in hydrocarbon pipelines. These regions may serve as a reservoir for recontamination of the system, and particular attention needs to be paid to the detailed design of the system. Once a bactericide program has been designed and implemented, any changes to the water sources, process design, or operation must be reviewed for its possible impact on the effectiveness of the bactericide program. Even small changes in a process plant can have a significant impact on microbial growth, so bactericide treatment should be seen as a constantly evolving and developing integrated program.

Inadequate Testing and Monitoring

To define the best bactericide regime for a particular system at any given time, competing products must be screened and tested in a realistic manner. Even before a product is tested for its effectiveness, it must be checked to ensure that it is compatible with the system. The basic requirement of any bactericide challenge test is to use a wide range of organisms freshly isolated from the system in an effort to recreate a relevent consortium. Performance evaluations must be undertaken on multispecies biofilms, with final evaluations performed on a limited number of products with sessile bacteria grown in special laboratory biofouling equipment. Such tests can indicate the best concentration and contact time but rarely allow assessment of the best interval between treatments. This interval can be determined most effectively in the field by using sidestream biofouling equipment to generate a more representative biofilm, with competing treatments injected into separate sidestreams, and by assessing the rate of regrowth of the biofilm. Field evaluation also allows verification of laboratory tests under actual system flow conditions.

It can take many months to identify a suitable bactericide regime, and it is clearly important to monitor the system so that its effectiveness can be confirmed. An adequate monitoring program should focus on the sessile population using sidestreams or in-line microbial monitors. Planktonic monitoring is suitable for assessing the degree of contamination in the system and to identify long-term trends, but such data can rarely be used to quantify the effectiveness of an individual bactericide batch treatment. Regular sessile sampling should take place before and after treatments and the results reviewed to give early warning of any reduction in performance of the bactericide. The monitoring program should be tied into the treatment program so that additional treatments can be given when bactericide effectiveness declines. This will also enable a further bactericide selection program to be commenced at an early stage, so that a new regime can be employed before the old bactericide fails.

NOVEL BIOFOULING CONTROL STRATEGIES

Conventional bactericide treatment regimes often fail to control bacterial biofilm populations; this has stimulated the development of new strategies to prevent biofouling or to slow its growth. Apart from methods to apply bactericide in a more efficient way, it is possible to manipulate the microbial population and suppress the problem without recourse to toxic compounds. Although these ideas work effectively in the laboratory, only a few new technologies have been used in field applications. Perhaps the main obstacle to the acceptance of new microbial control strategies is the possibility of unforeseen negative consequences on the system. It is therefore important to evaluate any new treatment program for unwanted effects such as enhanced biofouling and plugging, reduction in process plant efficiency, and increased general corrosion.

Nitrate

Nitrate (in the form of calcium nitrate or sodium nitrate) is routinely used in wastewater and sewage treatment industries to control nuisance smells caused by the growth of SRB. Nitrate suppresses biogenic sulfide production by encouraging the activity of nitrate-reducing bacteria, denitrifying bacteria, and nitrate-reducing and sulfide-oxidizing bacteria. Four major mechanisms have been proposed to account for this rapid inhibition of sulfide by nitrate-utilizing bacteria (NUB): outcompetition of SRB by NUB for organic nutrients, production of toxic intermediates such as nitrite, biological oxidation of sulfide by nitrate-reducing and sulfide-oxidizing bacteria, and switching SRB from sulfate to nitrate reduction (Nemati et al., 2001a). It is likely that all these mechanisms operate to various degrees in different environments, but the details of the interactions between NUB, SRB, and environmental conditions in reservoirs are not well understood.

Nitrate is one of the most widespread of the newly developing microbial control strategies in oil fields, showing much promise as a bactericide replacement. Much effort has been concentrated recently to develop effective nitrate-based downhole chemical treatment regimes to control SRB activity, limiting the rate of souring development. Nitrate addition to injection water is also being increasingly used to control microbiologically influenced corrosion, again minimizing the use of conventional bactericides. Full-scale and application trials have been carried out on numerous oil reservoirs and water injection systems (for example, Sunde et al. [2004] and Larsen et al. [2004]), with evidence of significant reductions in microbial corrosion, SRB activity, and hydrogen sulfide souring, accompanied by an alteration in the microbial community after long-term treatment (Voordouw and Telang, 1999).

Although nitrate treatment can be very effective for souring and microbial corrosion control, it is unlikely to control biofouling; this aspect may be of great concern in many systems. There is also recent evidence that nitrate can contribute to microbial corrosion (Voordouw et al., 2002; Dunsmore et al., 2004), so the negative aspects of this type of treatment must be assessed to ensure that unwanted side effects do not occur.

Nitrite

Nitrite is another simple inorganic compound that has been used to inhibit SRB activity and reduce the souring potential of a system. Nitrite is a specific inhibitor of SRB, and it also chemically scavenges preexisting sulfide. Treatment of oil and gas wells with nitrite has resulted in significant and rapid reduction in H_2S, as well as the virtual elimination of SRB in water samples. Beneficial effects of the nitrite used as a souring control technology have been observed for 3 to 7 months after the initial treatment (Sturman and Goeres, 1999). In oil production systems, similar effects (reduction in H_2S and SRB for extended periods) were noted, with a reduction in the corrosion rate also being measured. Nitrite is one component of biocompetitive exclusion technology, which uses a defined inorganic bacterial nutrient supplement to encourage a beneficial microbial population to inhibit sulfate reduction. This technique has been used successfully in oil and gas well squeezes (Hitzman and Dennis, 1998) and can be customized for specific applications. While biocompetitive exclusion has been shown to be successful in both laboratory and field applications, current research suggests that the microbial consortium does not change as a result of the addition of short-term nitrite or molybdate (Nemati et al., 2001b).

Molybdate

Molybdate ion is a specific metabolic inhibitor preventing sulfate reduction, and there are synergistic effects when molybdate, nitrate, and nitrite are combined (Percival, 1999). While molybdate is an efficient SRB inhibitor in laboratory culture studies, its effectiveness is very dependent upon the activity state of the bacteria. When SRB are in a slow-growth phase, sulfate reduction is stopped with 25 mg of molybdate/liter, whereas pure cultures in a rapidly growing (exponential) state tolerate up to 95 mg of molybdate/liter. Nemati et al. (2001b) showed that, for mixed field enrichments, 470 mg of molybdate/liter was required to inhibit sulfate reduction. It thus appears that the appropriate molybdate treatment will be very dependent on the growth rates of SRB in a system, and it is therefore not yet possible to establish a universal molybdate treatment strategy, since each system must be evaluated individually, particularly with respect to the activity of the SRB.

Anthraquinone

Low concentrations of derivatives of 9, 10-anthracenedione (commonly known as anthraquinone) inhibit respiratory sulfate reduction in laboratory tests of various cultures of SRB (Cooling et al., 1996). The proposed mechanism involves the uncoupling of ATP synthesis from electron transfer reactions, thereby preventing sulfide production. Further work on the mechanism of action confirmed that anthraquinone acted as a redox uncoupler, that it was inhibitory to sulfate reduction but not aerobic respiration, that it did not react with biofilm components, and that it had low toxicity to higher life forms. Anthraquinone becomes incorporated into the biofilm, and this gives longer lasting control of SRB in biofilms. Field case histories have been published (Burger, 2004) where sulfide and total suspended solid concentrations were reduced by using anthraquinone dosing of water injection and produced water injection systems, with a reduced use of conventional bactericides. The concept of using specific biostatic inhibitors (particularly if they target SRB in biofilms) offers more environmentally acceptable control measures than conventional toxic bactericides.

Sulfate Removal

Ceramic nanofiltration membranes can be used to reduce the concentration of sulfate in process streams. A full-scale plant (treating up to 390,000 barrels of water per day) can reduce the sulfate concentration from ca. 2,800 mg/liter in the influent seawater stream to ca. 40 mg/liter in the desulfated process stream. This technology has been applied to oil field water injection systems, primarily for the elimination of barium sulfate scaling problems; however, low sulfate concentrations also limit the activities of SRB, and therefore there are potential benefits for the control of microbial corrosion and reservoir souring (McElhiney and Davis, 2002). The most significant application is for the control of reservoir souring during seawater injection; in this case, SRB activity is limited by the availability of organic nutrients, not sulfate. A significant reduction in the sulfate concentration of injected water reduces the amount of sulfide that can be produced by SRB, leading to reduced biofouling, souring, and microbial corrosion.

Dispersant Technology

Multifunctional water treatment products based on filming amine technology exist, featuring low mammalian toxicity and rapid degradation for minimal impact on the environment. These chemicals act as a biofilm-slime dispersant rather than as a bactericide, migrating to and forming a film upon the wetted surfaces of the system being treated. This film prevents biofilms from forming on system surfaces and is thus effective in limiting the impact of bacterial growth on a system. Such treatments retard microbial population growth by preventing surface growth. Dosage consists of injection of low concentrations of the product for a short period of time each day to renew the film. Such treatments work best on clean systems where a good amine film can form; biofouled systems would probably need to be physically or chemically cleaned prior to the initial treatment, but an advantage would be that subsequently the scraping frequency could probably be reduced.

Biofilm dispersal through the addition of signaling molecules (such as HSLs) is a nascent yet promising area of current investigation. Dispersion of biofilms of the plant pathogen *Xanthomonas* has been promoted with small diffusible signaling molecules (Dow et al., 2003), and the importance of *N*-acyl-HSLs in biofilm accumulation and removal has been noted by several researchers (Puskas et al., 1997; Davies et al., 1998). Biofilm dispersal can also be brought about through the enzymatic degradation of extracellular polymer (Boyd and Chakrabarty, 1994). While research work and specialized application (mainly clinical) continues for these methods of biofilm removal, biofilm removal via enzyme or signal molecule addition is not available at this time for field application in the petroleum industry. Multiple-species biofilms and complex, highly variable water chemistries complicate the application of these techniques to industrial use at present.

Biofilm Inhibition by Immunoglobulin Treatment

One of the human immunoglobulins (immunoglobulin A) has been shown to inhibit the attachment of bacteria (including SRB) to steel surfaces in the laboratory (Videla et al. 2004), and this technology currently has application in the medical field to prevent biofouling of human implants. Such molecularly based technologies are likely to be impractical and not cost effective for many years, but the development of biotechnology will undoubtedly see applications for long-term prevention of biofouling problems in industrial systems.

Pulse Treatment

Batch treatment of bactericide treats less than 5% of the water passing through the system, and continuous treatment at effective concentrations is unlikely to be justified, due to the high chemical cost. Alternative strategies have therefore been developed to deploy the same amount of chemical in a different way to provide quasicontinuous treatment. An example of this is the Pulse treatment (Nalco-Exxon Energy Chemicals, 1996). A special pumping arrangement is used to automatically inject bactericide at a high concentration for a short period of time on a very frequent basis (for example, 1 to 2 min every hour or 30 s every 5 min). In this way, the system is continually challenged with the chemical so that bacteria are constantly exposed to an effective concentration, albeit for a short period of time. Such regular treatments are therefore more likely to maintain control of bacteria in the system and inhibit the regrowth of bacteria commonly seen with batch treatments. Pulse treatment has been applied to large systems, and tetrakis hydroxymethyl phosphonium sulfate treatment with this technique has been shown to maintain control of bacteria in water injection systems and reservoirs (Larsen et al., 2000).

Emulsion-Based Bactericide Deployment

Internal corrosion of hydrocarbon-carrying pipelines usually takes the form of pitting corrosion in the six o'clock position. It is most severe towards the end of the line and in low-lying regions (where stagnant water is trapped and biofilms develop), associated with welds and underneath deposits. In severe cases, the individual pits may penetrate the pipe wall, leading to an escape of oil and significant costs in term of cleanup, repair (or even replacement) of the line, and deferred oil revenues. Many oil pipelines are routinely treated with a film-forming corrosion inhibitor to protect metal from general corrosion due to water accumulation, yet these treated lines can still suffer pitting corrosion. Microbial corrosion occurs beneath deposits (waxes, silt, and biofilm) accumulating along with the water, despite corrosion inhibitor application. Control of SRB and microbial corrosion is especially difficult in an oil pipeline, since bacterial growth can be very localized and there is no continuous water phase where bactericidal chemicals can be dosed. Conventional treatment involves the application of a water-soluble, hydrocarbon-dispersible bactericide as a batch treatment with the oil stream, in an attempt to build up a bactericidal concentration in any water pockets that have collected in the line. Some of the disadvantages of using a water-soluble, hydrocarbon-dispersible bactericide may be overcome by using bactericides deployed in a water-in-oil emulsion. By having bactericide within the internal phase of this emulsion delivery system, control of bacteria within oil pipelines (and, potentially, oil reservoirs) could be more effective than conventional treatments, with the potential to allow transport of the bactericide to the end of the oil production line and diffusion into any available water present in the line.

Past, Present, and Future

Across the many growth environments, chemical conditions, and media present in petroleum extraction and refining, unchecked microbial growth is a ubiquitous and persistent problem. It is now recognized that biofilms represent the vast majority of this growth, far outstripping planktonic organisms in terms of both overall numbers and adverse impact. Advances in our understanding of biofilm processes have come at a rapid pace over the past 2 decades, with the application of new techniques which have elucidated the complex community composition, structure, and interactions, yielding a new understanding of interactions at the cellular level. Translating these advances into the development of practicable and effective biofilm monitoring and control technologies has been much slower. Despite all this effort in biofilm research over the last 20 years, oil field biofouling control measures remain focused on application of traditional chemical technologies and physical removal. Effective control of biofilm-related problems has proved to be exceptionally difficult, and eradication is virtually impossible in oil field systems, even with the most intensive biofilm control and monitoring programs. The recognition of biofilms as a major cause of infection in the medical context has focused research attention and funding into this area: the challenge for the 21st century is to translate the major advances in medical biofilm research into a simple, cheap, and effective strategy for minimizing the impact of biofilms in industrial systems.

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