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Contents

Up Front

Editorial

Technical Articles

Pipelines

Underground storage tanks

General interest

NACE News

Departments

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Bacterial biofilms in relation to internal corrosion monitoring and biocide strategies☆

J. W. Costerton,* G. G. Geesey,** and P. A. Jones***

This paper is a review of leading research in the field of bacterial corrosion monitoring, with specific emphasis on systems that transport liquids rather than gases. The primary mechanism of bacterial corrosion of metal surfaces involves the creation, within an adherent biofilm, of local physiochemical "corrosion cells." The practical consequence of this perception is that it is now known that bacteria must be in sustained contact with a metal surface, in well-organized microbial communities before the corrosion process is initiated. The detection of corrosion problems by monitoring planktonic sulfate-reducing bacteria (SRB) and the assessment of biocide efficacy by kill data on these planktonic organisms have led to an enormous waste of money. Noncorroding systems have been treated, and biocides have been applied to problem systems in totally ineffective concentrations and dosage strategies. Corrosion monitoring must be based on the detection of sessile corrosion bacteria (predominately SRB), and the only valid criterion of biocide efficacy must be the control of killing of these sessile bacteria within their structured sessile biofilm communities.

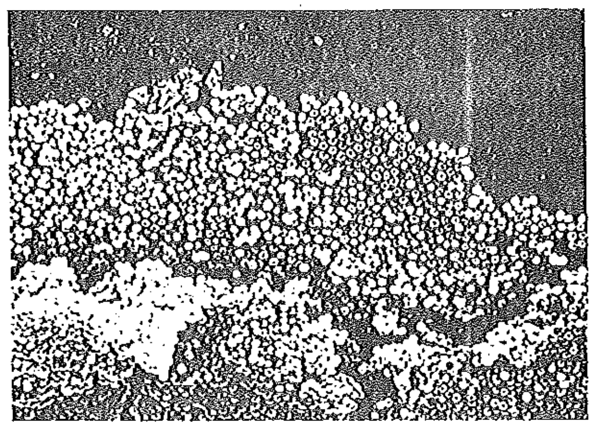

FIGURE 1 — Scanning electron microscopy (SEM) of the surface of stainless steel colonized by a single bacterial species in a laboratory experiment. The bacteria form thick biofilms on the metal surface and produce an amorphous polysaccharide matrix (lower right) that buries and protects the adherent cells.

Introduction

BEFORE THE REALIZATION THAT THE MAJORITY of bacteria in aquatic systems actually live in thick slime-enclosed biofilms on available submerged surfaces, students of bacterial corrosion speculated that products of bacterial metabolism exerted corrosive effects on metals without the necessity of sustained bacterial contact with these surfaces. Now that modern methods allow the detailed study of bacteria actually growing on surfaces, the development of adherent biofilms can be followed (Figure 1), and transmission electron microscopy (TEM) can be used to study the community structure within these biofilms (Figure 2), in which well-developed microcolonies of different bacterial types are observed [Figures 3(a) through (c)]. This new perception allows the visualization of a colonized metal surface with adherent microcolonies of different bacterial types juxtaposed against this surface within a complex adherent biofilm.

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Bacterial corrosion occurs only within the biofilm

The matrix, which encloses the cells of each of these microcolonies within the biofilm and mediates their adhesion to the metal surface and to the rest of the biofilm, is typically a highly hydrated anionic polysaccharide polymer containing large amounts of various uronic acids.[2] Geesey and colleagues have shown that the deposition of two different anionic polysaccharide polymers on adjacent areas of a metal surface is sufficient to cause corrosion, even in the absence of bacteria.[3] Little and colleagues have measured actual corrosion potentials between adjacent areas of a metal surface colonized by different types of bacteria.[4] Bacterial biofilms develop in all aquatic systems,[5] and the inner areas of biofilms (eight to ten cells thick) become anaerobic, even in aerobic systems [Figure 3(A)]. As anaerobiasis is established in the inner reaches of a thickening biofilm, anaerobic bacteria, such as sulfate-reducing bacteria (SRB), find themselves in a very favorable environment, and their replication gradually produces microcolonies of sister cells next to the metal surface [Figure 3(B)].

It is known that local pH differences of 1.5 pH units can be sustained between the centers of adjacent bacterial microcolonies, and data from Geesey, et al. and Little, et al. allow the speculation that local corrosion potentials develop between adjacent microcolonies, based on differences in the charge and metal-binding capacity

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FIGURE 2 — TEM of part of the bacterial biofilm on a colonized surface. Bacteria of the same type form microcolonies [e.g., (A) and (B)], and physiochemical differences between adjacent microcolonies can produce a local corrosion potential.

of their microcolonial matrix polymers [Figure 3(C)]. Some microcolonies do not remain monospecific but develop into consortia in which the primary colonizer attracts a secondary colonizer by its metabolic products, and this metabolic consortium often functions to pump substrate molecules and/or protons away from the colonized metal surface.

The end result of these natural ecological processes within the adherent bacterial biofilm is to produce a wide variety of foci on the metal surface that are different from neighboring foci in important physiochemical parameters [Figure 3(C)], and corrosion proceeds by conventional *corrosion cell* processes. This concept of bacterial corrosion explains the focal nature of this process (Figure 4) in which deep pits develop. These pits are filled with bacterial cells and the dehydration condensed residue of their polysaccharide matrices. Additionally, the requirement for a highly organized bacterial community within the adherent biofilm on metal surfaces may explain the effectiveness of *pigging* and other mechanical scraping procedures in corrosion prevention.

Our studies of biofilms in natural aquatic systems indicate that 10 to 14 days are usually required to reach climax populations and fully developed community structures, and a profound mechanical disturbance of the community at frequent intervals would surely retard the initiation of complete focal effects such as bacterial corrosion. This concept of focal bacterial attack on metals is closely paralleled by new data on similar focal bacterial attack on tooth enamel[6] and crystalline cellulose,[7] in which microcolonies of bacteria within adherent microbial biofilms exert their full physical and chemical effects on local surface areas.

One inescapable corollary of this concept of bacterial corrosion is that corrosion will not occur, even in the presence of numerous planktonic SRB, if these organisms have been unable, for ecological reasons, to set up effective microcolonies on the metal surface. A second corollary is that corrosion will continue unaffected even if planktonic SRB and even the SRB in the outer layers of the biofilm have been killed by biocides, provided that corrosive microcolonies continue to function in the biofilm directly adjacent to the metal surface. A third corollary is that a profound mechanical disturbance of the biofilms will retard bacterial corrosion and enhance the penetration and effectiveness of biocides very significantly.

Costly errors inherent in planktonic corrosion monitoring

Planktonic and sessile bacterial populations exist in all aquatic systems. Having studied natural systems for decades,[8] environmental microbiologists have concluded that the planktonic population, at any given time, is much smaller than the sessile population, and that it is never representative of the types of bacteria present in the attached sessile biofilms. The small planktonic population of bacteria constitutes the random and highly variable shedding (Figure 5) of the much larger and more complex sessile population, but this is what is contained in the random liquid *grab* samples that are routinely taken from pipes and vessels to detect a bacterial corrosion problem. For this reason, the species of cells found in any particular planktonic pipeline sample may not represent the predominant species within the sessile corrosion-causing biofilm (Figure 6).

It has been shown that oil pipelines whose water phase contained as many as 1.4×10^6 SRB/mL had fewer than 10 SRB/cm^2 in the vigorous biofilm population on the pipe wall [Figure 6(A)].[9] In other pipelines, sessile biofilm development was negligible in certain areas of the pipe wall [Figure 6(B)].[9] Conversely, sessile populations as high as 9.5×10^5 cells/cm^2 were recorded where planktonic samples yielded small numbers of SRB [Figure 6(C)] or no SRB at all [Figure 6(D)].[9] Because the numbers of corrosion-causing sessile SRB actually on the pipe wall cannot be deduced from planktonic samples, operators depending on these data have sometimes undertaken extensive biocide treatment of systems in which the pipe walls were entirely uncolonized by these organisms, and they have failed to treat systems that were actually under concerted microbiological attack.

Direct detection of sessile SRB populations is mandatory in the monitoring of metal systems for the presence of corrosion-causing bacteria, and devices have been developed to facilitate this process.[8] The systematic use of sessile sampling devices allows the operator to determine the extent of sessile SRB colonization at multiple points in a particular system and to base his decision on whether to treat with biocides on this rational database.

A practical example of the use of sessile bacteria sampling devices involved the installation of 8-in. (200-mm) pipe spools in the outlet lines of 12 oil tanks. Each pipe spool contained eight to ten Robbins Device studs.[10] The spools could be rotated so that the studs were placed on the bottom of the line for colonization and then moved to the top of the line for convenient retrieval.

Complex bacterial biofilms developed on the stud surfaces within six weeks in this system, which contained less than 1% water. The planktonic SRB populations in samples obtained from all 12 lines ranged from 1.6×10^4 to 1.4×10^6 cells/mL. The sessile bacteria samplers showed that in three of the lines, the biofilms that developed contained no SRB at all.

Biocide treatment of these three systems was suspended, and downstream sessile bacteria sampling sites continued to exhibit very low numbers of SRB. During the following 2.5 y of this study, intermittent biocide dosing of the other nine systems was sufficient to suppress the development of sessile SRB populations in all but one case. In this simple case, biocide treatments at a concentration sufficient to kill the sessile SRB alleviated the problem. Control was maintained by intermittent biocide treatment.

Thus, the systematic monitoring of sessile SRB populations in an oil system allowed the detection and effective treatment of a persistent corrosion threat without increasing expenditures on biocide.

Assessment of biocide effectiveness

When planktonic sampling is used to detect corrosion-causing bacteria and to assess their killing by biocides, a very costly series of errors is set in motion because unprotected planktonic bacteria

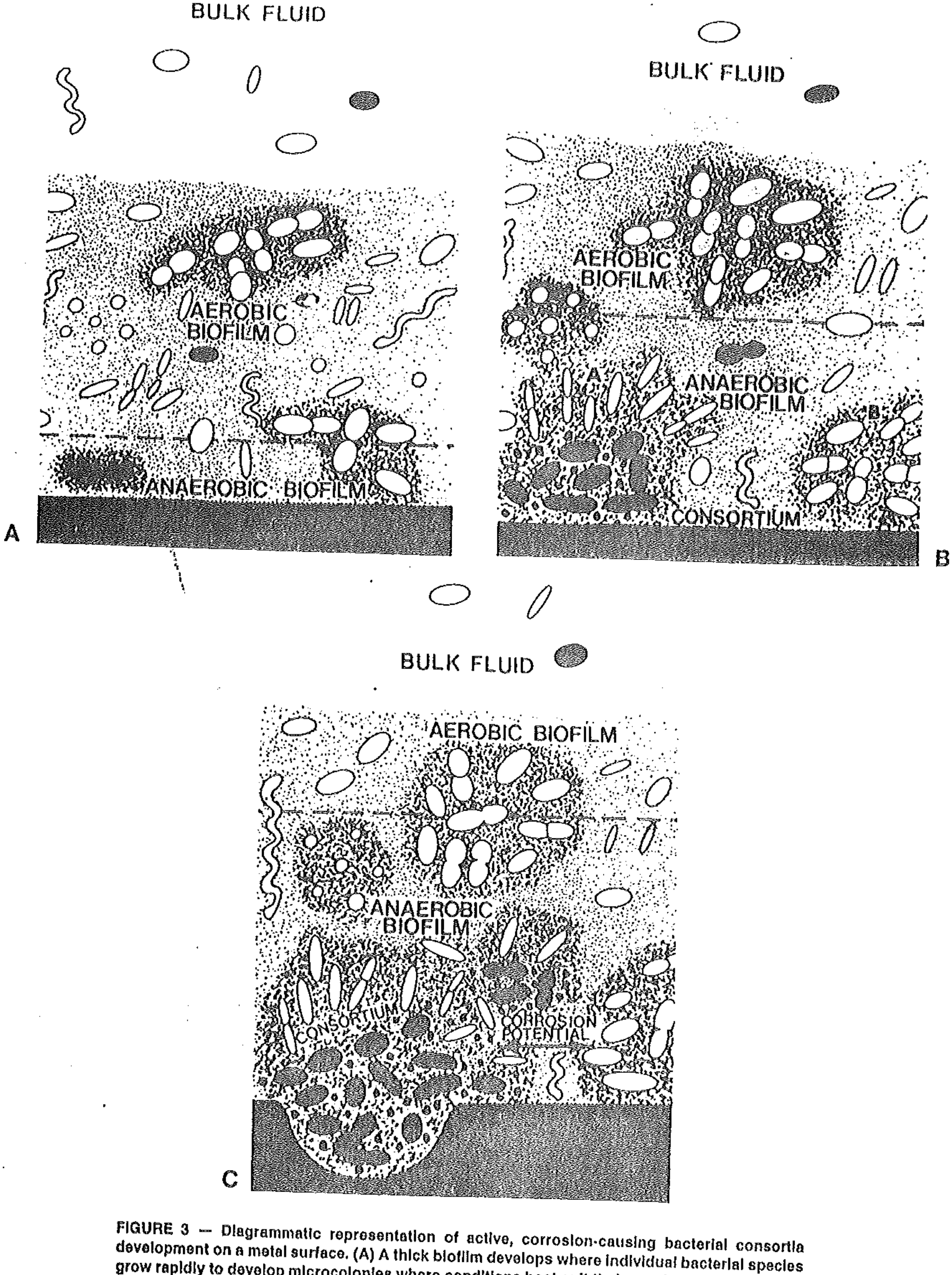

FIGURE 3 — Diagrammatic representation of active, corrosion-causing bacterial consortia development on a metal surface. (A) A thick biofilm develops where individual bacterial species grow rapidly to develop microcolonies where conditions best suit their requirements. When the biofilm attains sufficient thickness to exclude and consume oxygen, an anaerobic zone develops adjacent to the colonized metal surface. (B) SRB (black) and other anaerobic species develop simple microcolonies (right) and complex consortia (left) within which protons and metal cations may be transported or trapped to produce local chemical and physical differences at the metal surface. (C) An actual corrosion potential is established between specific areas on the metal surface, and metal is mobilized and deposited as in an electrochemical corrosion cell. As the corrosion pit deepens, the corrosion-causing organisms come to occupy it.

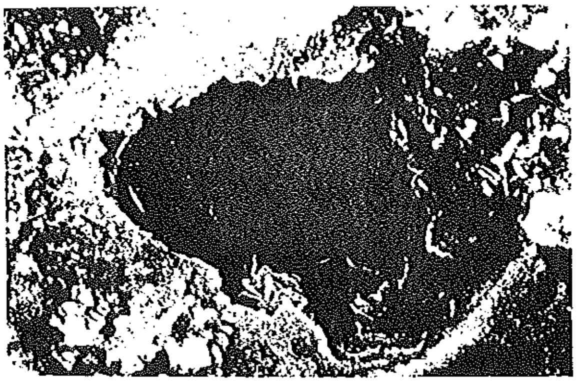

FIGURE 4 — SEM of the surface of a mild steel stud from which the biofilm had been removed by scraping. Note the well-developed crystal-filled *pit* that is characteristic of bacterial corrosion.

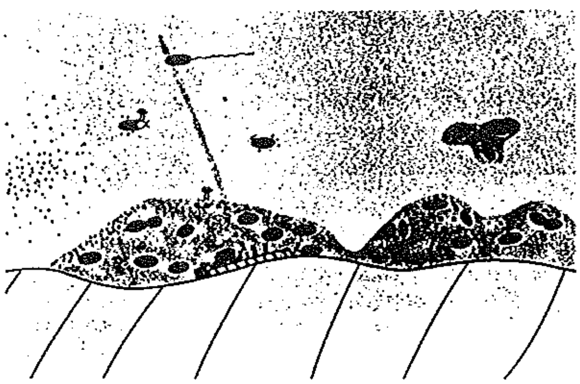

FIGURE 5 — Diagrammatic representation of sessile and planktonic bacteria in the aquatic system shows biocide-sensitive, swimming and floating, planktonic cells and biocide-protected sessile organisms within the extensive bacterial support matrix of the biofilm.

[Figure 6(E)] are very easy to kill,[10] and positive planktonic biocide kill data give the operator the comfortable but illusory idea that bacterial corrosion is under control. In practice, the corrosion of the system by sessile bacterial consortia continues, and live SRB are usually detected in the planktonic phase soon after biocide treatment is suspended. The use of biocides in reaction to planktonic bacterial data has cost the industry billions of dollars in ineffective biocide application. Because it is sessile biofilm bacteria (predominately SRB) that actually cause the bacterial corrosion of metals, the detection of bacterial corrosion problems must be based on sessile sampling, and the assessment of biocide efficacy must also be based on sessile sampling to indicate the complete control of corrosive species within the biofilm.

The experimental use of the Robbins Device[10] to test the efficacy of oil field biocides against both planktonic and sessile bacteria revealed that concentrations of these biocides sufficient to kill planktonic organisms had little effect on sessile bacteria [Figure 6(E)]. The biocides were chosen at random, were used as supplied to the oil field operation, and were used in the concentrations listed by Smith.[10] Colonized *studs* and flowing water were removed four and eight hours after dosing with the biocide, and it was obvious that the planktonic bacteria had been controlled effectively by all biocides at all concentrations used, but that the sessile bacteria were largely unaffected by these antibacterial agents.

While Smith records the comparative effects of commercial biocides on aerobes (aerobic plate count) and SRB,[10] the same relative sensitivities were observed for anaerobes (anaerobic plate count), and the total number of bacteria (live or dead) was determined by direct epifluorescence counting. The apparent comparative efficacy of specific biocides varied in different systems, but the differential sensitivity of the planktonic and sessile populations was always observed, and the sessile bacteria were always much more resistant.

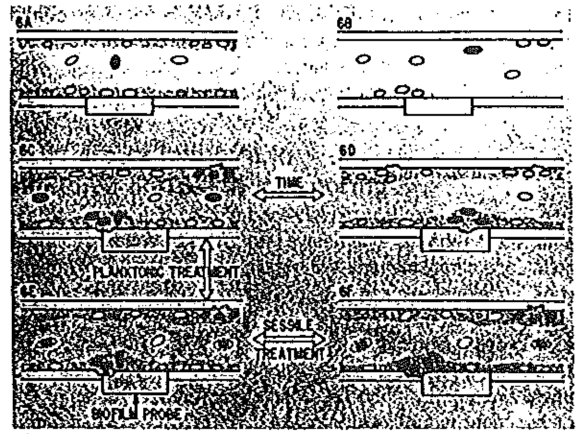

FIGURE 6 — Diagrammatic representation of planktonic and sessile bacteria (SRB are black) in a variety of different pipeline systems. (A) SRB are present in the planktonic population, but the biofilm that has formed contains none of these organisms. (B) SRB are present in the planktonic population, but biofilm formation is intermittent, and there are no sessile SRB. (C) and (D) SRB are present in the sessile biofilm population, but depending on the pattern of their release, they may (C) or may not (D) be present in the planktonic phase. (E) Treatment with an ineffective concentration of biocide kills the planktonic bacteria but leaves sessile SRB alive, so that corrosion continues. This situation would be detected by sampling the biofilm probe but not by a planktonic sample. (F) Treatment with an effective concentration of biocide for a suitable contact time kills both the planktonic and sessile bacteria, and this complete efficacy is detected by sampling the biofilm probe.

Perceiving that a truly successful biocide must penetrate the biofilm and kill the innermost sessile bacterial [Figure 6(F)], a careful and exhaustive study was undertaken using oil field-produced water and a large *battery* of Robbins Devices.[9] The oil field-produced water was obtained from a constant source and was changed daily as the Robbins Devices[10] were being colonized. All determinations of aerobic bacteria (by plating) and SRB (by the *most probable number* method) were conducted in triplicate, and the colonization of a control system was monitored by analyzing *studs* at frequent intervals.

The data obtained in this study clearly indicated that high biocide concentrations and extended contact times can produce complete killing of both anaerobes and SRB in these treated systems. Thus, it is concluded that the biocide efficacy in systems subject to bacterial corrosion can be established only when the actual corrosion-causing bacteria within the sessile biofilm population have been shown to be killed. If the control of these protected sessile populations is incomplete, the sessile consortia quickly recover, and live planktonic bacteria quickly reappear when treatment is suspended.[10]

Essentially, the bacteria within sessile biofilms grow slowly because of substrate limitations and their extensive production of extracellular polysaccharides and because the complex consortia of physiologically associated species that actually cause bacterial corrosion are similarly slow to develop.[11] For these reasons, the complete killing of sessile SRB populations initiates a cycle of dead biofilm sloughing, recolonization, biofilm development, and consortium development, so it may take weeks or months to reestablish effective corrosive bacterial consortia.[9]

Biocide development

There is now overwhelming evidence from medical,[14] environmental,[13] and industrial[10] microbiology that planktonic bacteria differ profoundly from sessile bacteria in their susceptibility to antibacterial agents. It is fair to say that most commercial biocides were developed and tested for their ability to kill planktonic bacteria in the laboratory and the field. Some biocides were tested against sessile bacteria early in their development (notably, the isothiazalones), and recent careful tests of these compounds for the ability to penetrate biofilm and bacterial debris and actually kill sessile bacteria indicate that they are cost effective in the systems tested.[9] It is anticipated that a new generation of penetrating biocides will be developed, based on their demonstrated ability to kill sessile biofilm bacteria in heavily fouled systems, and these new compounds will be very effective in corrosion control.

Summary

Modern research in the bacterial corrosion field has produced unequivocal evidence that this process is a function of organisms growing in biofilms adherent to the metal surface. Much remains to be discovered concerning the precise molecular mechanisms of this focused corrosion process, but it is concluded that immediate juxtaposition of SRB and other corrosion-causing organisms to the metal surface is a prerequisite of bacterial corrosion.

Planktonic sampling is inherently misleading in the detection of bacterial corrosion because the presence of SRB in planktonic samples does not reflect the presence of a consortium of SRB in the sessile biofilm, and the absence of SRB in planktonic samples does not reflect the actual absence of these organisms in the adherent biofilm. However, planktonic data are most misleading when they are used to claim the efficacy of a particular biocide treatment, and operators must begin to draw the correct conclusion from these data; i.e., SRB in the planktonic phase at the time of sampling have been killed.

Sessile bacterial sampling detects SRB within the biofilm on the metal surface and identifies real corrosion problems, and sessile bacteria kill data reflect the control of the bacteria actually corroding the metal surface. The use of modern methods and equipment to obtain accurate sessile bacterial samples will facilitate the detection of actual bacterial corrosion problems, the development of effective biofilm-penetrating biocides, and the effective use of these agents and of mechanical techniques to control this costly and ubiquitous natural process.

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