MICROBIALLY INFLUENCED CORROSION AND FILTER PLUGGING - DON'T YOU WISH THEY WERE EASY TO DIAGNOSE?

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biofouling, filter-plugging, microbes, MIC, filter biodeterioration, filter fouling, coaleser fouling, biofilm, corrosion

ABSTRACT

Root cause analysis is the process used to identify the fundamental cause for an undesirable condition. Premature filter failure due either to plugging or other mechanism is generally perceived to be an acute problem. Despite the waxing and waning of their symptoms, systemic microbial infections are chronic. Understanding the nature of microbially influenced corrosion and microbially mediated filter failure can facilitate better system monitoring and maintenance processes and system design.

This presentation reviews the fundamental mechanisms of microbially influenced corrosion and premature filter failure. It also provides guidelines for diagnosing microbially mediated problems in petroleum product systems ranging from crude oil production to waster-based metalworking fluids.

INTRODUCTION

Throughout the petroleum industry, cost-of-quality discussions are frequently conflictual and left unresolved. Though generally perceived as acute, filter plugging is really a chronic cost-of-quality problem. Moreover, filter-plugging is sometimes indicative of another chronic problem: microbially influenced corrosion or MIC.

The causes of premature filter plugging include many conditions that have no apparent link to biological activity in petroleum systems. However, as I'll discuss during this presentation, microbes in remote parts of a petroleum system play critical, unrecognized roles in the symphony of events that lead to premature filter failure.

Historically, MIC was the acronym for microbially induced corrosion. By the early 1980's, researchers realized that microbial communities often played secondary or lower-order roles in corrosion processes. Conveniently, the acronym could be preserved, while it's meaning changed rather substantially.

Today, I shall offer some working definitions for both filter failure and MIC. I'll then suggest an approach for diagnosing microbially influenced filter failure and corrosion. I shall use the term petroleum product(s) throughout this presentation, since the general principals I'll be discussing in this presentation are equally applicable to crude oil, liquid fuels, lubricants and water-based metalworking fluids.

MICROBIALLY INFLUENCED CORROSION

As I implied during my introductory comments, our understanding of MIC has changed considerably since von Wolzogen Kuhr and van der Vlugt proposed an anaerobic corrosion model in 1934 [1]. As with much of our understanding of ecological processes, our current MIC model is considerably more complex than the one originally proposed. Figure 1 provides a schematic model of the various roles of microbes in ferrous metal corrosion.

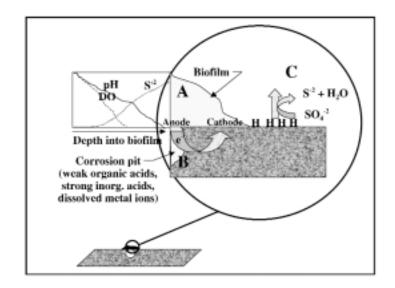


FIG. 1. BIOFILM FORMS PHYSICAL BARRIER
CONDUCTIVE TO ELECTROPOTENTIAL GRADIENT
(GALVANIC CORROSION CELL). MICROBIAL
ACTIVITY WITHIN BIOFILM CRATES PH AND
CHEMICAL GRADIENTS AS ILLUSTRATED N PH,
DISSOLVED OXYGEN (DO) AND PH PLOTS AS
FUNCTIONS OF DEPTH WITHIN BIOFILM. B.
WITHIN PIT, ACIDS AND OTHER METABOLITES
ACCUMULATE, ACCELERATING RATE OF IT
FORMATION. C. HYDROGEN THAT WOULD
NORMALLY ACCUMULATE AT THE CATHODE IS
BIOLOGICALLY SCAVENGED BY SRB AND
OTHER ANAEROBES, THEREBY PREVENTING
PASSIVATION.

MIC may be physical, chemical or a combination thereof. As soon as pioneering microbes attach to metal surfaces, electropotential (galvanic) gradients form. These are driven by the surface REDOX potential differences between the exposed and covered surfaces. Within 24 to 48 hours, biofilm microcolonies have matured sufficiently so that the physicochemical conditions within the biofilm are substantially different from those of the surrounding fluid. Although the bulk petroleum product may contain water in the parts per million range, biofilms are mostly water.

Starkey [2] and other early investigators believed that the sulfate reducing bacteria (SRB) were the primary bacteria involved in MIC. We now understand that SRB participation in the process was overestimated. Aerobes (bacteria and fungi that require oxygen) and facultative anaerobes (bacteria that can thrive whether or not oxygen is available) generate organic acid wastes. Although these are weak acids, their protons can react with chloride, nitrate and sulfate ions to form hydrochloric, nitric and sulfuric acid, respectively. In contrast to the weak organic acid metabolites, these strong acids can attach metals aggressively. Microbially generated acids also partition into petroleum products, thereby increasing product corrosivity. Some biodeteriogenic microbes routinely recovered from petroleum product systems depend on the petroleum product as their food. Hydrocarbon and nonhydrocarbon constituents provide all of the nutrients these microbes need to thrive in these systems. Other microbes (for example the SRB mentioned above) depend on organic chemicals that the hydrocarbon degraders secrete as wastes (metabolites).

Typically, microbes don't work as pure cultures (one type of microbe^a) in industrial systems. Instead, consortia form within biofilms. Several different types of microbes work together to mediate changes that none of the individuals could. One example is the manner in which facultative anaerobes create an environment suitable for SRB growth. The facultative anaerobes scavenge oxygen and convert complex organic molecules in to simpler ones that the SRB can eat. Without the facultative anaerobes, the SRB wouldn't have a suitable habitat. Recent research at the University of Montana^b and elsewhere has demonstrated that biofilm ecology is much more complex than microbiologists had originally thought. Cells within the biofilm differentiate. Similar to what happens in higher organisms^c, cells from individual types of microbes take on very different characteristics depending on their position within the biofilm. Not only are different microbes working in concert, but also individual types of microbes are behaving as though they were genetically different types of microbes.

The biofilm's gross structure is also quite complex. Although they appear to be just amorphous masses of slime, biofilms are highly structured, with pores and channels for transporting nutrients and eliminating toxic metabolites. In many respects, a mature biofilm resembles a simple multicellular organism. Small wonder that we are barely

scratching the surface in terms of understanding MIC.

For our current discussion, the critical issue is that ferrous and ferric hydroxide and ferrous sulfide are insoluble in either petroleum products or water. Thus iron particulates abraded from MIC sites are transported to filters where they may be the dominant retained material. It's not just a matter of SRB. Nor it is a simple question of recovering microbes from bulk fluid samples. Since the microbes are predominantly localized within biofilms, significant infections may go unnoticed until after systems fail.

FILTER FAILURE

Until a few years ago, I defined filter failure as either: a) the loss of filter integrity such that it no longer retained particles; or b) unacceptably low flow-rates, high pressure differentials or both, My remarks today will focus primarily on this second class of failures. However, I will also speak to third type of filter failure about which I've become concerned only recently. In this third scenario, coalescing filters fail to retain water or particulates although flow-rates and pressure differentials remain unchanged.

FILTER PLUGGING

Before continuing, I'll offer two more operational definitions. I define premature filter plugging as filter plugging that occurs surprisingly early. The second term, filter-failure really applies only to the case of integrity loss. Premature filter plugging generally is not really filter-failure. It's a symptom of other system or fluid problems. Filters can restrict flow prematurely if the medium is incompatible with the fluid being filtered. Presuming that compatibility has been addressed adequately during the filter selection process, premature plugging occurs when the filtered fluid's particulate load is atypically high.

No doubt you have all seen slimed-up filters, either directly or in photographs. Lest I disappoint, I offer figure 2.

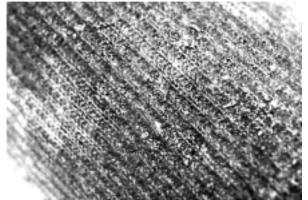


Photo courtesy of H.L. Chesneau, Fuel Quality Services, Inc. Flowery Branach, GA.

Fig. 2. Sock filter coated with bioslime.

The photo illustrates a filter that has failed due to slime accumulation. Conventional microbiology yielded approximately 108 CFU¹/cm². Metalworking fluid filters and some distillate fuel filters will give you colony count yields in this range. For many years, I considered plate counts from

filter media as a convenient way to recover viable microbes from systems with not easy access to internal surfaces (figure 3a).

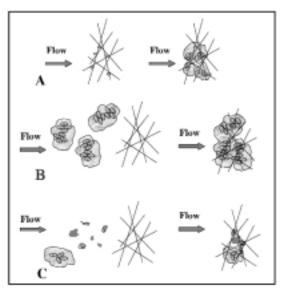


FIG. 3. FILTER PLUGGING MECHANISMS.

A. MICROBES GET TRAPPED WITHIN FILTER MATRIX, WHERE THEY PROLIFERATE AND PRODUCE SLIME. PROBABLY A RARE EVENT.
B. FLOCS OF BIOSLIME, HAVING SLOUGHED OFF OF UPSTREAM SURFACES, ARE TRAPPED BY FILTER MATRIX. A COMMON EVENT. C. DETRITUS, INCLUDING POLYMERIZED PETROLEUM PRODUCT, INORGANIC DETRITUS AND BIOSLIME FLOCS ARE TRAPPED BY FILTER MATRIX, THEREBY PLUGGING IT. THIS IS PROBABLY THE MOST COMMON BIOLOGICALLY MEDIATED PREMATURE PLUGGING MECHANISM.

My experience in gasoline and non-water-based lubricants has caused me to reconsider filter media as an optimal sampling point. Microbes need water to proliferate. If conditions within the filter medium are anhydrous, microbes won't colonize the medium. Since most petroleum product degrading bacteria are $<5~\mu m$ long (and $<1~\mu m$ thick), individual cells are likely to pass through filter media. Fungal filaments and yeast cells are larger (>100 μm diameter) and are more likely to be retained. However, they may be damaged beyond repair and thereby be rendered unable to produce colonies on selective growth media. All this means that bioslime development by microbes growing on or within the filtration medium may be a rare event.

But let's return to the biofilms I introduced while discussing MIC. Biofilms are dynamic structures. Biofilm material is routinely sloughing off and being regenerated. When fluid conditions change substantially, biofilms may disaggregate. This happens when you treat a heavily fouled system with a shock-dose of biocide. Great flocs of biofilm (including entrained microbes) break loose and either settle to the system's bottom, get flushed downstream to filters or both. This traveling slime plugs filters, as illustrated in figure 3b.

As I noted earlier, corrosion byproducts will also be transported to filter media, contributing to premature filter plugging. Moreover, biofilm material is very effective glue, enabling it to trap considerable amounts of inorganic and non-biological material. In fact, total elemental analysis of bioslime scraped off of system surfaces typically yields less than 5% combined carbon, oxygen and hydrogen. This remains surprising only until you consider the relative atomic masses of the organic elements (C, H, O, N, S & P) versus the inorganic elements that you are likely to find in slimes retrieved from ferrous systems (Fe, Zn, Ni, V, Co, Mo) as illustrated in Table 1.

		Inorganic Element	Atomic Mass
Organic Element	Atomic Mass		
Carbon(C)	12.0	Cobalt(Co)	58.9
Hydrogen (H)	1.0	Iron (Fe)	55.8
Nitrogen(N)	14.0	Molybdenum(Mo)	95.9
Oxygen (O)	16.0	Nickel (Ni)	58.7
Phosphorous (P)	31.0	Vanadium(V)	50.9
Sulfur(S)	32.1	Zinc (Zn)	65.4

TABLE 1. ATOMIC WEIGHTS; ORGANIC COMPOUND ELEMENTS AND ELEMENTS ASSOCIATED WITH FERROUS STRUCTURES.

Microbes are certainly not the only cause for filter plugging. They may not even be the predominant cause. However, since inorganic filter residues can eclipse biomass, even when the problem is biological. Moreover, biodeterioration process symptoms often overlap non-biological deterioration symptoms. Consequently, the incidence of microbially influenced problems may be grossly underestimated.

In summary, microbes may colonize filter media thereby causing premature filter plugging (figure 3a). This is probably a rare event. Flocs of bioslime embedded with lots of inorganic material can become entrained onto filter media thereby causing premature filter plugging (figure 3b). This type of event may account for 20 percent of all premature filter-plugging incidents. Depending on nominal pore-size, filter media will trap biodeterioration byproducts (such as corrosion byproducts and oxidized or polymerized petroleum-product material) (figure 3c). Although this type of filter residue may not include biomass, biological processes (or reaction with biomolecules) mediated its formation. This type of event may account for 30 percent of all premature filter-plugging incidents. Non-biogenic particulate loads probably account for the remaining 50% of all premature filter-plugging incidents.

COALESCER FAILURE

Recirculating turbine lubricants pick up condensate water and particulate contaminants. In-line filtration units are used to remove these contaminants using a combination of coalescence and particle retention. As the particle cake approaches maximum loading, pressure differential builds and lubricant flow plummets. Occasionally, however, coalescing filters fail before there is any significant pressure differential change. Gross observations of filter cross-sections fail to yield any apparent failure cause.

Several years ago, I speculated that low-level bacterial infections might be changing the physicochemical properties of the coalescer fibers. Recoverable populations on the order of 102 to 104 CFU/cm² wouldn't affect fluid flow, by could produce sufficient biosurfactant to change fiber wettability characteristics. Although biocide treatment effectively inhibited the filter failure symptoms, I haven't had the opportunity to develop any direct data with which to test my model more thoroughly. However, research on the adverse effect of diethyleneglycol monomethyl ether (DiEGME) on jet fuel coalescer performance provides concepts that I think that we can reasonably extrapolate to support the biodeterioration model.

At each of the last two International Conferences on Stability and Handling of Liquid Fuels, Spencer Taylor and his colleagues have presented his initial and refined models for monofiber coalescer function [3, 4]. Coalescer monofilaments are hydrophilic and are bonded with hydrophobic resin droplets. As fuel (or any other low to moderate viscosity petroleum product containing dispersed water) flows through the coalescer, water adheres to the hydrophilic fibers, forming droplets. As a droplet moves along the fiber, in the direction of the fluid flow, it assumes a teardrop shape. Hysteresis (the difference between the leading and trailing contact angles; (A and (R, respectively) and interfacial tension between the petroleum product and water phases ((OW) create a retention force (Fr) (figure 4):

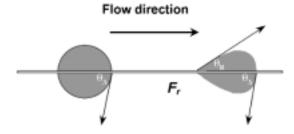


Fig. 4. Fluid retention force.

As droplet moves along hydrophilic filament, retention force, F_r increases with the difference between the leading contact angle, θ_a and the trailing contact angle θ_R . F_r is also determined by the filament diameter, d_f , and the interfacial tension between the petroleum product (γ_{OIL}) and water phases (γ_{OW}) .

Fr = $\pi d \gamma_{ow} (\cos \theta_R - \cos \theta_A)$ (from Taylor [3].

Where \mathbf{d}_f is the fiber's diameter. As water droplets move along hydrophilic resin fibers, they grow. At some point droplet size exceeds its hydrodynamic stability limit and it falls out of suspension. High Fr facilitates droplet size growth and, consequently, coalescer performance. Taylor has demonstrated that Fr decreases as surfactant (DiEGME) concentration increases. Biosurfactants, produced in situ, can

be expected to have the same effect as DiEGME on Fr. This is one mechanism by which low-grade infections can contribute to coalescer failure.

Now let's return to the hydrophobic resin beads that are used to bind the coalescer's fibers. Hydrophobic regions of the coalescer medium act as barriers to water droplet growth. This facilitates water droplet release. If zwitterionic biomolecules coat the resin surfaces the biomolecules will make the surfaces hydrophilic. When this happens, the resin beads no longer function as droplet barriers. Thus microbes contribute to a second filter failure mechanism. Figure 5 illustrates how low-grade microbial infections can contribute to coalescer failure.

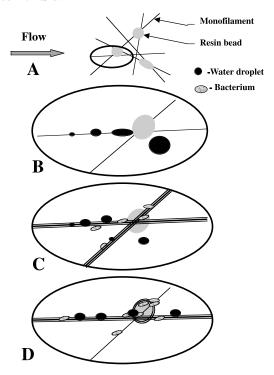


FIG. 5. SPECULATIVE MODEL: MICROBIALLY MEDIATED COALESCER FAILURE MECHANISMS.

A. SCHEMATIC SECTION OF COALESCER FILTER. HYDROPHOBIC RESIN DROPLETS BIND HYDROPHILIC MONOFILAMENTS. B, C & D SHOW REGION WITHIN ELLIPSE IN A. B. NORMAL FUNCTION. HIGH WATER-OIL INTERFACIAL TENSION AND LOW CONTACT ANGLES ENABLE WATER DROPLETS TO GROW TO CRITICAL SIZE DURING FINITE CONTACT PERIOD ON FILAMENT. RESIN BEAD FUNCTIONS AS BARRIER, FACILITATING COALESCENCE. C. MICROBES HAVE PRODUCED SUFFICIENT BIOSURFACTANT TO DECREASE INTERFACIAL TENSION SIGNIFICANTLY. DROPLET SIZE GROWTH IS RETARDED AND COALESCER FUNCTION DIMINISHED. D. MICROBIAL COLONIZATION OF RESIN BEAD HAS RENDERED ITS SURFACE HYDROPHILIC. BEAD NO LONGER FUNCTIONS AS DROPLET BREAK (COALESCER SCHEMATIC FROM TAYLOR [3]).

DIAGNOSIS

SAMPLING

In his Life of Johnson, Boswell [5] recounted an exchange between a woman of the British nobility and Samuel Johnson, the famous, 18th century lexicographer. Upon being introduced to Mr. Johnson and realizing that he was the author of the recently published dictionary, the noblewoman expressed her outrage at having found dirty words in his volume. Mr. Johnson replied that if she had found such words she must have been searching for them.

Our situation is often just the opposite. The most elegant analytical techniques will inevitably fail to provide useful information if they are performed on inappropriate or mishandled samples. Rather than attempt to provide an encyclopedic review of sampling procedures, I focus on offering a few guiding principals.

Before collecting your sampling supplies, it's imperative to review the system's history. Review engineering documents, operational and maintenance records and make some direct observations of the problem system. Estimate where, within the system, you are most likely to find corrosion hot spots. Although filter-plugging may have been the symptom that launched the investigative effort, the processes that caused filter-plugging were occurring elsewhere in the system. If process fluids are likely to stratify or change properties as they pass through the system, you'll want to collect representative samples of each fluid state (for example bulk, near-bottom, pre-filter, post-filter and post-engine return fuel in a ship's diesel fuel system).

Dead-legs, sumps and other relatively quiescent zones are all places where sediment and biomass are likely to accumulate. Interfaces are the best place to look for biomass. In systems containing water immiscible liquids (fuels and lubricants) biofilms are likely to develop at fluid-to-fluid interfaces. Tank and piping surfaces often support active biofilms. Consequently, surface scrapings can be particularly useful for diagnosing biodeterioration problems. Corrosion coupons can provide particularly useful information about corrosion or biodeterioration processes within the system. Place and seal intact filters into plastic bags for transport to a diagnostic facility.

Fluid samples should be collected in clean glass jars. Samples vulnerable to photooxidation may be placed in brown glass bottles. Generally, placing clear glass bottles into cardboard boxes and keeping them away from light is sufficient to prevent significant photooxidation. Materials samples are best protected from confounding reactions (air contact, etc.) by storing them immersed in the medium to which they were in contact. For example place a fuel-line corrosion coupon in a jar filled with fuel from that line.

Take precautions to avoid contaminating samples as you collect them. Also be sure to label each sample and document each sample's identity in a sampling log. Details regarding the exact location, within the system, from which the sample was

taken, date and time of sampling, identity of the person collecting the sample and initial observations may be critical to successful root cause analysis.

TESTING

As with sampling, the test battery you select may affect you ability to complete a successful root cause analysis. The level of effort spent on defining the testing strategy is miniscule relative to the cost of misdiagnosis. I offer a few guidelines for testing. If you have followed my pre-sampling recommendations, you have probably started developing one or more hypotheses about the problem's root cause. Quite possibly you've compiled a list of IF-THEN statements. The IF portion of each hypothesis suggests your first echelon tests or you first order diagnostics. The THEN portion of each hypothesis suggests the next echelon of testing.

Different root causes may create similar symptoms. Consequently you need to consider the various known processes that can create each symptom. Second and higher echelon testing should then focus on differentiating amongst alternative root causes.

Be certain that you have sufficient, representative sample material to permit at least three testing echelons.. I recommend four types of tests: gross observations, physical, chemical and microbiological. Refer to ASTM D-6469 [6] for a list of specific test methods in each of these categories. Little et al. [7] have created a very readable guide on diagnosing MIC. I haven't come across a similar guide for diagnosing the root cause of filter plugging, although ASTM D-6469 addresses fuel system biodeterioration.

Certain sample properties are perishable. They change rapidly over time. Different test methods include directions for inhibiting change during storage. Sample preservation procedures differ depending on the parameter to be measures. Consequently, it's often necessary to split samples so that subsamples may be preserved appropriately. In particular bottom-water parameters including dissolved oxygen, sulfide, pH, alkalinity (or acidity) and microbial contaminants (qualitatively and quantitatively) begin changing as soon as the sample is drawn. Minimally, fluid sample dissolved oxygen and sulfide concentrations should be determined as soon as possible, preferably within minutes after collection. Viable recovery, pH and alkalinity/acidity tests should be performed within six hours after sampling.

INTERPRETATION

FILTER FAILURE

Filter failure root cause analysis can be particularly challenging. The most fundamental data (volume filtered, initial and terminal flow rates, etc.) may not exist. Moreover, premature plugging may be caused by special causes of variation or processes that are beyond the control of the organization contending with the plugging problem. Symptoms and their causes may be intermittent. When filter plugging is caused by microbial activity within the immediate

system, successive filters will be more likely to plug prematurely. If biodeterioration byproducts have been introduced from upstream distribution channel stages, filter plugging will only occur until the offending material has been removed. Depending on the contamination load, and product turnover and settling rates, this may require one or more filter changes.

More evidence for a biological cause of filter plugging is more likely to be found in the filtered fluid than on the filter medium. Biofilms sloughing off of system surfaces or liquid-liquid interfaces will cause downstream filter plugging. Chemical differences between near-bottom and bulk fuel samples indicate biodeterioration is significant. Microbial activity will deplete lower molecular weight molecules (carbon-number) and enrich the fuel for larger molecules. As discussed above, microbial activity will make bottom-water more corrosive.

When coalescers fail without accompanying pressure differentials, suspect biodeterioration. Since the concentration of biosurfactant is likely to be below detection limits, assess the impact of biocide treatment. Use only hydrocarbon or universally soluble, U. S. EPA antimicrobial pesticides that are approved for use in lubricants. Perhaps future research will make better diagnostic test methods available.

MICROBIALLY INFLUENCED CORROSION

Diagnosis of MIC is best based on direct observation of the infected site. Typically, MIC is characterized by pitting. Barnacle-like tubercles often cover MIC pits. Rust orange external crust covers a blackened interior. Corrosion patterns may be as telling as pit geometry. Lines of pits along the bottom dead-center of pipes or along the typical fuel-water interface line in fuel tanks exemplify MIC.

The take home lesson here is to try to think as inclusively as possible while developing root cause hypotheses. Avoid the natural tendency to discard data that don't fit proposed hypothetical models. Assume that sampling, testing and interpretation will often be iterative. Initial results may be cryptic. When this happens, identify the types of additional data that could help decrypt the initial results. Collect the requisite additional data and repeat the interpretation process. You may have to repeat this cycle several times to unveil the true root cause.

CONCLUSIONS

Although MIC and filter plugging may seem to be disparate topics, they are often linked. MIC generates byproducts that are subsequently retained by filter media. Moreover, microbial slimes, in dynamic equilibrium on system surfaces, contribute to both MIC ecology and filter burdens.

Microbes influence corrosion by creating electropotential gradients between adjacent regions of metal surfaces. Biogenic weak acids attack metals directly. More significantly, they also react with inorganic salts to produce strong acids that are very aggressive. The enzyme hydrogenase present in sulfate reducing bacteria and certain other anaerobes scavenges hydrogen ions from the cathodic termini of electrolytic corrosion cells. This depassivation process accelerates corrosion rates.

Although bioslimes may develop as microbes grow within the matrix of filter media, this is a rare event. More often there's insufficient water entrained in filter media to support growth. Slime recovered from filter media has been transported and deposited there from upstream. Filter plugging due to slime build-up can be considered a direct effect. Polymeric oxidation products and MIC debris may also cause filter plugging as secondary effects of biodeterioration.

I have speculated that low-grade infections can change surface chemistries within high-tech filter media. When this happens, filter functionality degrades without the traditional symptoms of plugging.

Testing and diagnosing both MIC and filter plugging is complex and requires thorough planning. Perhaps the greatest challenge to successful root cause analysis, when MIC or filter plugging occur, is obtaining a thorough history of the events preceding the symptoms. Biogenic and non-biogenic factors create many similar symptoms. Consequently, I recommend an echelon approach to root cause analysis.

Premature system component failure due to MIC and premature filter failure both represent considerable costs-of-quality. Moreover, in many applications they can be safety risks. Learning to recognize and control microbial contamination in industrial process systems can reap both economic and workplace safety improvements.

Footnotes

- a Microbial taxonomy differs from higher organism taxonomy. Microbes have fewer fixed physical characteristics. Bacteria are currently characterized into four major categories that are further divided into a total of 35 groups. The primary bacteria we encounter in petroleum products are all in Category I, Groups 2 (aerobic...gram-negative bacteria), 4 (gram-negative, aerobic...rods and cocci), 5 (facultatively anaerobic gram-negative rods) and 7 (...sulfate...reducing bacteria). Within each group, taxonomically unique organisms are sub-classified into genus, species, strain and biovariation. The later two echelons are not always used. Despite the continued use of cell shape and gram-stain reaction, most taxonomic classification depend on batteries of several hundred physiological and biochemical tests, including DNA homology (genetic matching) and lipid profile testing. This is a far cry from looking under a microscope and identifying the microbe observed.
- b Go to their website at http://www.erc.montana.edu/ for a complete listing of research papers on this topic.
- c As an embryo develops, somatic cells differentiate into the specialized cells that comprise different types of tissue.

- d unacceptability is defined operationally on a case by case basis, and can range from < 10 percent to nearly 100 percent flow restriction.
- e I'm surprised if a filter plugs earlier than halfway though it's expected performance life. For example if a filter is designed to process 0.5 million gallons and plugs ((50% flow-reduction) at 200,000 gallons, it has failed prematurely.
- f CFU colony forming units: the number of colonies that form when you apply a sample to a solid growth medium.
- g For example: IF there is an invert-emulsion zone between the fuel and water layers of my fuel tank bottom sample, THEN biodeterioration is a likely cause; or IF the filter residue is primarily comprised of small, granular rust particles, then somewhere upstream there was flash corrosion and subsequent palling.

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